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13. ABSTRACT ***(Maximum 200 Words)***

Twenty fetal sheep were exposed to impulses with peak levels of 169 dB SPL (pSPL). Auditory evoked potentials and behavioral state were recorded from the fetuses before and after impulse exposures. In the uterus of pregnant sheep, the pSPL varied as a function of fetal head location. When the fetal head was against the abdominal wall, peak levels were within 3.7 dB of airborne levels. When the fetal head was deep within the uterus, the peak amplitude decreased by 18.5 dB. Data from ten fetuses exposed at 117 days gestational age (dGA) and from 10 fetuses exposed at 127 dGA revealed significant elevations in post-exposure auditory brainstem response thresholds for low-frequency eliciting stimuli. Scanning electron microscopy revealed damage to inner and outer hair cells located in the middle and apical turns of the cochlea. Cochleae of fetuses exposed at 117 dGA showed similar amounts of damage when compared to cochleae of fetuses exposed at 127 dGA. Recordings of behavioral state indicated disruption of normal cycling during and shortly after the exposure. Significant changes occurred for fetal heart rate, and spectral power in the electrocorticogram for Alpha, Delta and Theta bands. The major finding was that exposure to 20 impulses at 169 dB peak sound pressure level produced significant damage to primarily the inner row of hair cells located in the apical region of the cochlea in fetal sheep.

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INTRODUCTION

The overall aim of this research was to determine if high-intensity impulses provoke changes in hearing and sleep of the fetus *in utero*. The purposes of this research project were to measure the transmission of impulse noises into the uterine environment and to evaluate the effects of exposures delivered to the flank of pregnant sheep on the hearing, inner ear histology and sleep-state of the fetus. Three discrete studies are reported: 1) impulse transmission into the abdomen of sheep (N=10); 2) effects of impulse exposure on auditory evoked potentials and cochlear histology (N=21); and disruption of electrocortical activity of fetuses in response to impulses (N=6).

BODY OF THE FINAL REPORT

Background of Previous Research

The relation between exposures to intense sound and decreases in hearing sensitivity of adult male workers was first reported in the early 18th century. Since then, noise-induced hearing loss (NIHL) has been widely studied. The permanent and handicapping effects of intense noise on adult hearing have been well documented. Recently, attention has shifted to the possibility of NIHL during fetal life (Gerhardt, 1990).

Significant numbers of American working women of childbearing age are noise exposed. The Committee on Hearing, Bioacoustics, and Biomechanics (CHABA, 1982), attempting to protect fetal hearing, suggested that pregnant women avoid noise exposures greater than 90 dB(A). Other investigators believed that these recommendations could needlessly exclude women from the work force (Niemtzow, 1993). There is a paucity of evidence to support either conviction. Two retrospective studies (Lalande et al., 1986; Daniel and Laciak, 1982) found increased risk of hearing loss in children with occupationally noise-exposed mothers. But these studies have been criticized for methodological shortcomings (Niemtzow, 1993; Henderson et al., 1993).

Findings from experimental animals have been paradoxical. Dunn et al. (1981) exposed pregnant sheep to intense steady-state noise for 4 hours a day, 5 days a week for several weeks. Thirty to forty days after the lambs were born, ABR thresholds were normal. The ABR, a far-field recording of a bioelectric response to sound from the auditory mechanism, is a common clinical and research hearing assessment tool. In contrast to the Dunn study, Griffiths et al. (1994) measured the ABR from fetal sheep *in utero* before and after a single 16-hour, broadband noise exposure (100 Hz to 10 kHz) at 120 dB sound pressure level. The investigators found significant changes in ABR thresholds and latencies immediately following noise exposure, although these changes were temporary. In a related study (Gerhardt et al., 1999), the fetal sheep ABR was recorded over a 23-day period following a similar noise exposure delivered at 113 days gestation (gestation for sheep is 145 days). No immediate changes in ABR thresholds were found, but thresholds for the noise-exposed group were significantly higher than for an age-matched, nonexposed group after 2 weeks or more. Cook et al. (1981) demonstrated ABR Wave IV latency differences between guinea pigs exposed to textile noise during the last trimester of gestation and a non-exposed control group.

In most instances, postnatal noise exposures occur in air. Prenatally, externally generated

sounds must pass from air to the fluid medium of the uterus in order to reach the fetus. The intrauterine sound environment is dominated by frequencies below 0.5 kHz (Gerhardt et al., 1990). Externally generated sound transmission to the fetal head is more efficient for low frequency, steady-state sounds than for higher frequency, steady-state sounds. Low-frequency sound pressures penetrate the uterus with little reduction in level and higher frequencies are reduced by about 20 dB. Transmission of impulse noise to the fetal head has not been measured, although it would be expected to follow the same pattern.

Methods, Assumptions and Procedures

During sterile surgery, the instrumentation for chronic recording of the evoked potentials was implanted in two groups of sheep, either at a gestational age of 115 days or 125 days. The fetus was exteriorized and the fetal head prepared for evoked potential and behavioral state recordings. A hydrophone was sutured near the fetal head in some preparations. The purpose of the hydrophone was to record acoustic levels in the intrauterine environment during the impulse noise exposure.

Two days after surgery, the ewe was placed in a sound-treated booth and fetal evoked potential thresholds were assessed using tone bursts and clicks. Behavioral state and heart rate were assessed for at least one hour before exposure in the older fetuses. After pre-exposure testing, ewes were exposed to 20 impulses produced by a shock tube. A second hydrophone, connected to one channel of a spectrum analyzer and positioned close to the maternal flank, recorded the pressure-time history generated by the shock tube. A simultaneous recording (channel 2) from the hydrophone *in utero* was obtained. Measurements from the two hydrophones were used to calculate transmission characteristics from air to the fetal head. The post-exposure evoked potentials were followed for 20 days in fetuses exposed at 117 days gestational age and for 10 days in the fetuses exposed at 127 days gestational age. At 137 days gestational age, the ewes and fetuses were sacrificed and cochleae removed and prepared for scanning electron microscopy.

Measurements of heart rate, electrocorticogram and electrooculography were simultaneously recorded on strip-chart paper and FM magnetic tape. These measurements were obtained one hour before exposure to 20 impulses, during exposure and for one-hour post exposure. The analog signals from the magnetic tape were subjected to spectral analyses in order to evaluate changes in behavioral state and heart rate responses produced by the stimulus.

We assumed that the impulses would be affected by transmission through maternal tissues and fluids, resulting in a reduction of peak sound pressure and a reduction in high-frequency spectral energy. We further postulated that fetal evoked potential thresholds would be elevated for low-frequency stimuli following exposure to impulses and scanning electron microscopy would reveal hair cell loss concentrated in the middle and apical turns. In addition, behavioral state cycling would be disrupted during and for a period of time immediately after the exposures.

Results and Discussion

Effect of Intraabdominal Location on Impulse Characteristics. (Information in this section can be found in: Gerhardt KJ, Abrams RM, Huang, X, Griffiths SK and Peters AJM.

Intraabdominal sound pressure levels during impulse noise exposure in sheep. Military Medicine, In Press).

There are significant numbers of women of child-bearing age in the workplace (Sheehan, 1996), and it can reasonably be assumed that many are pregnant at any given time and may be exposed to significant levels of noise in their jobs. While hearing conservation programs are available for adults, there have been inferences (CHABA, 1982) that fetuses, whose hearing mechanisms have matured by the beginning of the third trimester (Hepper and Shahidullah, 1994), may be over-exposed to noise in the workplace.

Two retrospective studies found increased risk of hearing loss in children born to women who were occupationally noise-exposed (Lalande, et al., 1986; Daniel and Laciak, 1982). Studies of fetal sheep *in utero* confirm that intense, continuous noise exposures delivered to the ewe produce elevations in fetal auditory brainstem response thresholds (Griffiths, et al., 1994; Huang, et al., 1997) and create damage to the hair cells of the inner ear (Gerhardt, et al., 1999). Other reports present contradictory information and interpretations (Dunn, et al., 1981; Niemtzow, 1993).

In the vast majority of situations, noise exposures occur in air. Fetal exposure is an exception in that it occurs in a fluid environment. In order for continuous noise to reach the fetus it must first pass through the tissues and fluids surrounding the fetal head. Pressure variations produced in amniotic fluid stimulate the fetal skull and evoke an auditory response (Gerhardt, et al., 1996). The stimulus that reaches the fetal inner ear has been significantly filtered such that the low-frequency sounds (125-250 Hz) reach the inner ear with a loss of only 10-15 dB, whereas the higher frequency sounds are reduced by over 40 dB (Gerhardt, et al., 1992).

Although most industrial noise is continuous, impulse or impact noises are common in some occupations. Impulse noise includes all forms of high-intensity short-duration sounds, i.e., from impacts produced by industrial equipment to intense blast waves associated with military operations. Impulse durations vary from microseconds for small arms fire to hundreds of milliseconds for a sonic boom. Intensities for those signals may vary from less than 100 dB to over 185 dB peak sound pressure level (pSPL).

In addition to duration, the signature of the impulse wave also may vary. Impact noise, produced by one object striking another, is reverberant, has a relatively long duration and is usually typed as a B-duration wave. Impulses, produced by rapidly expanding gases, have very short durations and high peak levels often exceeding 150 dB. This class of impulse is a type A-duration wave. Type A-waves have very brief rise times and generally include greater high-frequency energy as compared to Type B-waves that have longer rise-times and more low-frequency energy.

The signature of an impulse wave can be further described in terms of its peak level (pSPL), rise-time and duration. Peak sound pressure level is defined as the highest pressure in dB at the onset of the impulse (Coles, et al., 1968). Rise-time is the time taken for the single pressure fluctuation that formed the initial positive peak to increase from ambient to the peak pressure level. Duration can be calculated in two ways depending upon wave type. For A-duration pressure waves, duration is the time required for the initial pressure wave to rise to its positive peak and return momentarily to ambient. For B-duration pressure waves, duration is the time

interval between impulse onset and the point in time at which the envelope decays by 10 dB from peak level (Smoorenburg, 1992).

The acoustic characteristics of impulse noises recorded from within the uterus are not known with certainty. Thus, a carefully controlled study of intraabdominal transmission characteristics of impulse noise seemed warranted. This was completed in four non-pregnant sheep exposed to 169 dB pSPL stimuli. The use of these animals was justified because sound transmission properties of non-pregnant sheep (Peters, et al., 1993) are similar to transmission properties in pregnant ewes (Vince, et al., 1985; Gerhardt, et al., 1990) as well as pregnant humans (Querleu, et al., 1988; Nyman, et al, 1991). Information from this study may have implications for pregnant women exposed to high-intensity impulse noises.

All experiments were carried out under the Guidelines for the Care and Use of Animals at the University of Florida. Four non-pregnant adult ewes were killed as part of other experiments. Wool was sheared over the abdominal and flank areas and along a four-inch swath running the full length of the spinal column. Ewes were then suspended horizontally from a metal frame according to the following procedures. Two punctures were made through the skin two inches apart at seven locations over the spine from coccyx to occiput. A neoprene coated, 18 gauge copper wire was inserted through one wound and tunneled two inches under the skin where it was brought out through the second wound. The seven wires were tied around the metal frame in such a way that the spine was parallel to the frame.

The frame and animal were then carried into a sound-treated booth whose inner walls had been covered with four-inch thick sound-attenuating material. The animal was positioned so that its left flank was four feet from one wall of the booth. In this wall was an exponential horn which was attached outside of the booth to a conventional shock tube. The exponential horn was designed to match the impedance of air at the end of the shock tube to the impedance of air within the sound-treated booth. The shock tube has an overall length of 10 feet and an inner diameter of four inches.

The rupture of a mylar diaphragm following a pressure build-up of 70 psi of nitrogen created an impulse noise of 169 dB pSPL in air. A miniature hydrophone (Bruel & Kjaer Instruments, Inc. [B&K], Naerum, Denmark, model 8103) was used to record the SPL of the impulse in air and a second hydrophone was used to record simultaneously the impulse in the abdominal cavity of the ewe. Hydrophones have a flat frequency response in air and in fluids (+/- 0.5 dB) from 0.1 Hz to 320 kHz and an omnidirectional sensitivity pattern. They were calibrated at 140 dB SPL (re:20 μPa) with a standard hydrophone calibrator (B&K type 4223).

The hydrophone was inserted into the abdominal cavity through a stab wound in the right mid-flank region (side of animal away from the horn). The hydrophone was pushed horizontally until it was felt against the wall of the left flank (nearest the horn). Sound pressure levels were recorded from this "proximal" position.

The hydrophone was then withdrawn along the axis of stimulation in order to place it midway between the proximal and distal (the point of entry on the right flank) positions, that is, deep in the abdomen. After recording from this position, the hydrophone was withdrawn so that it was just inside the abdominal cavity (distal position) where final measurements of the waveform were completed.

The outputs from the hydrophones were fed into the two channels of a frequency analyzer (B&K, model 2123) that used constant percentage bandwidth filters. Time waveforms were recorded simultaneously from both hydrophones. One hydrophone was positioned in line with the shock tube and directly above the proximal flank. The second hydrophone was in the abdominal cavity in one of the three locations described above (proximal, medial or distal). The time waveforms were saved to disk for off-line analysis of peak SPL, rise time, and peak duration. The waveforms were transformed into the frequency domain for analysis of the frequency content expressed in 1/3-octave bands.

Repeated measures analysis of variance (ANOVA) was used to test for an overall difference among response means (pSPL, rise-time and duration). Site was considered as a within-subject factor and the average of three replications considered as the response for each animal at each site. Tukey's multiple pairwise comparison procedures were used to maintain a significance level of .05 for pairwise comparisons among animals and sites.

Figure 1 includes representative waveforms of an impulse recorded with one hydrophone in air and a second hydrophone positioned in the abdomen. In this figure, the intraabdominal hydrophone was located very close to the flank of the ewe that was nearest the shock tube. The morphology of these two waveforms is similar and the peak levels differ by about 3 dB (169 dB pSPL compared to 167 dB pSPL in the uterus). Intraabdominal recordings at other locations showed marked differences in impulse morphology. Figure 2 includes recordings of an impulse recorded in air and the same impulse recorded with a hydrophone positioned deep within the abdomen. In this figure, pSPL differed by 23 dB.

Table 1 includes the average values from the four animals for pSPL, rise-time and duration. A judgment regarding the wave type is also included. In the proximal location, an A-duration pressure wave was noted in three of the four animals. In the fourth ewe, a type B-duration pressure wave was observed. The morphology of the pressure waves recorded in air was very similar to the morphology recorded at the proximal location, yet was distinctly different than the waveforms recorded at the medial and distal locations.

Table 1. See text above.

Location	pSPL	Type	Rise-time (ms)	Duration (ms)
Air	169.4	4-A	0.12	0.82
Proximal	165.7	3-A; 1-B	0.44	6.67
Medial	150.9	4-B	2.47	41.47
Distal	154.7	4-B	1.22	36.41

The ANOVA applied to pSPL values revealed significant differences as a function of hydrophone location ($F_{3\text{-}9}$=40.19; p=<.0001). Tukey multiple pairwise comparison showed that the average pSPL for air (169.4 dB) was essentially the same as that for the proximal location (165.7 dB), yet significantly different than the medial (150.9 dB) and distal (154.7 dB) values.

Evaluation of values for rise-time in milliseconds (ms) revealed results similar to those found for pSPL. A statistically significant main effect was noted ($F_{3\text{-}9}$=29.33; p=<.0001). Post hoc testing indicated that values for rise-time were not different when recorded in air and at the

proximal location. However, rise-time differed statistically between air recordings and those made at both the medial or distal locations. Proximal recordings of rise-time differed significantly from the recordings completed at the medial location but not the distal location.

The ANOVA applied to duration data revealed a statistically significant effect for location ($F_{3,9}$=11.77; p=<.0018). Post hoc testing revealed that measurements in air differed significantly from recordings obtained at the medial and distal locations. No differences were found between air and proximal recordings and between medial and distal recordings.

All pressure waveforms were transformed into the frequency domain and averaged as a function of recording location. Figure 3 displays the results of this process in 1/3-octave bands. A few observations warrant comment. First, the 1/3 octave band levels from 200-315 Hz are greater when recorded at the proximal location as compared to air. Enhancement of low-frequency sound pressure within the abdomen has been observed in other studies (Gerhardt, et al., 1990; Querleu, et al., 1988). The tendency for low-frequency enhancement to occur may relate to intraabdominal resonance.

Second, there is a noticeable drop in level above 500 Hz when comparing air to the proximal recording site. This drop, or attenuation, exceeds 10 dB at 5000 Hz and has been noted for steady-state noise and tones studies (Gerhardt, et al., 1990; Querleu, et al., 1988). Considerably greater attenuation for the high frequencies is seen for recordings at the medial and distal locations. Differences of 20-30 dB are seen in this figure between recordings in air and those at the medial location.

Peak SPLs recorded from within the abdomen were highly variable and ranged from 153 to 168 dB. Peak levels in air averaged 169.9 dB. The overall morphology of the waveforms related to pSPL and frequency content of the impulse. Peak levels recorded in the abdomen averaged 7.3 dB less than those recorded in air. Spectral analysis in one-third octave-bands revealed peak levels in air at 315 Hz compared to 160 Hz when recorded from the abdomen. As predicted from earlier studies, high-frequency sound pressures were attenuated by maternal tissues and fluids by up to 25 dB.

The intraabdominal position of the hydrophone influenced both pSPL as well as spectral distribution. When the hydrophone was near the abdominal surface, peak levels were approximately 3 dB less than the peak levels recorded in air. When the hydrophone was deep within the abdomen, the morphology of the waveform changed and peak levels were approximately 20 dB less than those recorded in air.

Compared to the wealth of information on mammalian responses to periodic motion, there have been very few studies of the effects of shock (Griffin, 1990). Most shocks result from displacement of the body or its parts when it is contact with solid objects. However, it is possible also to produce shocks with high-intensity airborne blasts. Few who have experienced a howitzer discharge would doubt that their whole bodies move perceptibly during this experience. Because of prohibitions against non-medical invasive procedures, it is not known in humans how such an airborne impact might affect individual parts of the whole body. This issue takes on military significance when one considers the transmission of vibratory energy through the abdominal segment, and how this exposure could effect the fetus, should the pregnant mother-to-be be on the firing line.

The findings that steady-state noise exposure to the fetus *in utero* affect inner ear has important implications for pregnant women (Griffiths, et al., 1994; Huang et al., 1997; Gerhardt,

et al., 1999). Impulse exposures of the levels used in this study have been reported to produce permanent cellular damage in fetal sheep as found in steady-state exposure studies (Gerhardt, et al., 1998). There are no compelling data to demonstrate that human fetuses have the same susceptibility to noise as do sheep, or that human fetuses are at risk to inner ear damage produced by noise levels to which pregnant women might normally be exposed. However, the data from this study warrant consideration in the formulation of guidelines that may be developed to protect the fetus of pregnant women from noise damage.

Electrophysiological Measures: Auditory brainstem response (ABR) and amplitude modulation following response (AMFR) thresholds were measured from each animal in both groups according to the experimental schedule. Thresholds for the ABR were defined as the lowest levels at which the response was observed in the waveform. Thresholds for the AMFR were defined as the lowest level at which the amplitude at the modulation frequency (50 Hz) exceeded the average amplitude at nearby frequencies (below 100 Hz) in an FFT-derived spectrum of the averaged brain activity. All threshold values were determined independently by two experimenters who then met to resolve any disagreements that became apparent in reviewing the results.

Two experimenters also measured latencies for the ABR independently, subsequently meeting to reach consensus on any disagreements. Electrophysiological thresholds and latencies then served as the dependent variables in repeated measures analysis of variance (ANOVA). The criterion of statistical significance (α-level) used for all analyses was $p \leq 0.01$. Significant main effects noted in the ANOVA were subsequently investigated using pair-wise comparisons yielding **t** statistics.

Thresholds. Figures 4 and 5 contain displays of electrophysiological thresholds from the early-exposed (figure 4) and late-exposed (figure 5) groups of animals. Developmental changes similar to those documented by Pierson, et al. (1995) may be noted, particularly in Figure 4. Hearing sensitivity (as measured electrophysiologically) improved in the early-exposed group most dramatically for the 1, 2 and 4 kHz tone bursts. Smaller declines in threshold are noted for clicks and the 500 Hz tone burst-evoked ABR. AMFR thresholds to 500 and 1000 Hz carrier signals also tended to decline during the period of the experiment. A small (5 dB) but non-significant elevation in 500 Hz AMFR thresholds from the pre-exposure to the post-exposure measures can be noted.

The reduction in threshold (improvement in sensitivity of the animal) is noted in the early-exposed group in spite of the introduction of the impulse exposures. This observation is further supported by the finding of significant reductions in electrophysiological thresholds in the later measurement periods from the early-exposed group.

Table 2 displays the significant ANOVA results for electrophysiological thresholds. There were no significant effects of measurement time among thresholds measured in the later-exposed group of animals. This is consistent with the observations of Pierson, et al. (1995), who noted much smaller developmental changes in threshold at later gestational ages. Once again, however, a small post-exposure increase in threshold is noted for the 500 Hz carrier AMFR and the 500 Hz tone burst ABR, suggesting a transient elevation in hearing sensitivity in this frequency range.

Table 2. Significant ANOVA results for electrophysiological assessments of thresholds.

Group/Stimulus	Effect		Statistic	df	p
Early					
	Stimulus		**F** =52.635	7,35	0.001
	Measurement Date		**F** =6.213	6,30	0.001
	Stimulus x Date		**F** =2.438	42,210	0.001
	Pair-wise				
Click	Pre	Rec 3	**t** = 6.094		0.001
		Rec 5	**t** = 6.047		0.001
		Rec 7	**t** = 5.000		0.002
1 kHz	Pre	Post	**t** = 3.094		0.009
		Rec 1	**t** = 3.811		0.002
		Rec 3	**t** = 6.326		0.001
		Rec 5	**t** = 6.351		0.001
		Rec 7	**t** = 4.636		0.002
2 kHz	Pre	Rec 1	**t** = 3.149		0.008
		Rec 3	**t** = 6.051		0.001
		Rec 5	**t** = 4.819		0.001
		Rec 7	**t** = 4.492		0.003
AM 500	Pre	Rec 3	**t** = 3.400		0.007
Late					
	Stimulus		17.853	7,28	0.001

Latencies. Auditory brainstem response latencies were significantly influenced by the time of observation and stimulus level in both experimental groups of animals. No significant interactions between time of measurement and stimulus level were observed. As would be expected, higher stimulus levels produced shorter response latencies. The significant main effects for time of measurement were evaluated using pair-wise comparisons. Figures 6 through 9 display the mean latency shifts across measurement time for the late-exposed group of animals in response to clicks and 4 kHz, 2 kHz and 1kHz tone bursts, respectively. Mean latency shifts for the early-exposed group of animals are displayed in figures 10 through 13. Tables 3 and 4 summarize significant pair-wise differences for time of measurement in the early- (Table 3) and late- (Table 4) exposed groups of animals.

Table 3. Significant pair-wise comparisons for latency measurements in fetuses exposed to impulses at 117 days gestational age (early-exposed group). Asterisks indicate significant differences.

Early Group	Post-exp		Rec 1		Rec 3		Rec 5		Rec 7	
Click	70	50	70	50	70	50	70	50	70	50
Pre-exp			*		*	*	*	*	*	
Post-exp					*	*	*	*	*	
Rec 1					*	*	*	*	*	*
Rec 3										
Rec 5										
4kHz	70	50	70	50	70	50	70	50	70	50
Pre-exp	*	*	*	*	*	*	*	*		
Post-exp					*	*	*	*		
Rec 1					*	*		*		
Rec 3							*	*		
Rec 5										
2kHz	70	50	70	50	70	50	70	50	70	50
Pre-exp		*	*	*	*	*	*	*	*	*
Post-exp			*		*	*	*	*	*	*
Rec 1					*		*	*	*	*
Rec 3									*	*
Rec 5										*
1kHz	70	50	70	50	70	50	70	50	70	50
Pre-exp					*	*	*	*		
Post-exp					*	*	*			
Rec 1					*	*	*	*		*
Rec 3										
Rec 5										

The notable trend in the early-exposed group is for latencies obtained at later gestational ages to be shorter than those obtained at earlier gestational ages. These results are consistent with those seen in the threshold measures. In the late-exposed group of animals, significant differences tended to involve the latest gestational ages, with a developmental trend toward shorter latencies at later gestational ages.

Table 4. Significant pair-wise comparisons for latency measurements in fetuses exposed to impulses at 127 days gestational age (late-exposed group). Asterisks indicate significant differences.

Late Group	Post-exp		Rec 1		Rec 3		Rec 5	
Click	70	50	70	50	70	50	70	50
Pre-exp							*	*
Post-exp					*	*	*	*
Rec 1								
Rec 3								
4kHz	70	50	70	50	70	50	70	50
Pre-exp							*	*
Post-exp						*	*	*
Rec 1							*	*
Rec 3								*
2kHz	70	50	70	50	70	50	70	50
Pre-exp								*
Post-exp							*	*
Rec 1							*	*
Rec 3								
1kHz	70	50	70	50	70	50	70	50
Pre-exp					*		*	
Post-exp					*		*	*
Rec 1								
Rec 3								*

Early- versus Late-Exposed Group Responses at the Same Gestational Age. The potential effects of impulse exposures were evaluated between groups by comparing electrophysiological measures in the pre-exposure condition in the late group to the recovery measure from the early group obtained at the same gestational age. The pre-exposure measure in the late-exposed group occurred at 126 days gestational age (dGA). This coincided with the fifth recovery measure from the early-exposed group (mean value at recovery 5 = 127.2 dGA). Electrophysiologic thresholds and latencies obtained at that gestational age were compared between groups using ANOVA. No significant differences in latency were observed between the two groups. Mean latency values

(plus and minus one standard error of the mean) are displayed in figure 14. Similarly no significant differences were observed in the electrophysiologic thresholds for the two groups.

Histology. The temporal bones were removed bilaterally and fixed with phosphate-buffered 2.5% glutaraldehyde and 2% paraformaldehyde mixture for 24 hours, and then decalcified with 15% EDTA (phosphate-buffered) for 10 days. Subsequently, the temporal bones were washed in phosphate-buffered solution, post-fixed in a 1% osmium tetroxide phosphate-buffered solution for one hour, and dehydrated in a graded series of ethanol up to 70% for micro-dissection. After dissection of the organ of Corti, the specimens were critical-point dried in 100% ethanol using liquid carbon dioxide, mounted on aluminum stubs, sputter coated with platinum to a depth of approximately 75 nm and examined with a field emission SEM. Serial photomicrographs of the entire length of the organ of Corti were used to construct a cochleogram for each cochlea.

Both the inner hair cells (IHC) and the outer hair cells (OHC) from undamaged regions of the organ of Corti had normal appearance with an orderly arrangement of stereocilia except for the apical region closest to helicotrema. The shape of OHC stereocilia resembled the classic "W" pattern, the IHC stereocilia resembled a "U", and the length of stereocilia decreased from apex to base as seen in other mammals (Gulick et al., 1989).

Following noise exposure, hair cell damage of both IHC and OHC was noted primarily in apical and middle turns of the cochlea. Abnormalities of hair cells included distorted and/or missing stereocilia, giant stereocilia and phalangeal scarring. Stereocilia of the IHC were often missing in the apical turn. Stereocilia of the OHC were missing, distorted, and in some cases broken apart. Also, giant stereocilia were observed in the apical turn. Although in some instances, stereocilia of IHC and OHC were relatively intact, cytoplasm ballooned out of the hair cells and at junctions of supportive cells (Figure 15). Ballooning of cytoplasm has been reported to arise from the apical end of the hair cells following noise exposure (Hunter-Duvar et al., 1982). In the middle turn, there was evidence also of phalangeal scarring, yet fewer distorted stereocilia were noted as compared to the apical turn (Figure 16). The IHC and OHC in the basal turn appeared intact with no evidence of cytoplasmic ballooning, phalangeal scarring or missing, distorted and broken stereocilia.

There appears to be a progression of change to the hair cells that ultimately ends with complete replacement of sensory epithelium by surrounding supporting cells. In some instances, a webbing formed over the tops of damaged stereocilia (Figure 17). Following what appeared to be a fairly rapid process, the stereocilia were absorbed and the webbing ended up lying flat on the apical end of the hair cell (Figure 18). The hair cell itself was completely absorbed and replaced with supporting cells, presumably Deiter cells (Figure 19).

Cochleograms were plotted for each cochlea following procedures described by Schuknecht (1953). Damaged or distorted stereocilia and presumed locations of missing hair cells were counted and mapped by location. Stereocilia were considered damaged if they were absent or altered in any way. Areas of damage are indicated as a percentage of hair cell loss as a function of distance along the organ of Corti. The individual cochleograms, as a function of gestational age at the time of the exposure, were averaged and plotted as mean cochleograms (Figure 20). Note that most hair cell damage found in the noise-exposed animals was confined to the middle

and apical turns. On average, the IHCs were more severely affected than the OHCs, primarily in the apical region of the cochlear duct between 5 and 20% of the total distance from helicotrema.

Scanning electron micrographs shown in this study revealed damage consistent with reports of inner and outer hair cell alternations following noise exposure in other species (Hunter-Duvar et. Al., 1982; Saunders et al., 1985). When lesions occur in most smaller laboratory animals, OHCs are the first to be damaged and end up as the most severely altered when compared to IHCs. In contrast, fetal sheep demonstrated greater damage on average to IHCs than OHCs. While not observed in most experimental animals, greater damage to IHCs than OHCs following noise exposure has been reported in rabbits (Engström and Borg, 1981). Evaluation of cellular damage from noise-exposed fetal sheep revealed a greater loss of OHC3 as compared to the other rows of OHCs. A similar finding has been reported in adult monkeys (Moody et al., 1978).

Fetal Behavioral State. (Information in this section can be found in: Bauer R, Gerhardt KJ, Abrams RM, Huang X, and Bauer K. Effects of impulse noise stimulation on electrocorticogram and heart rate in fetal sheep. Biology of the Neonate, Under Review).

The late-term human fetus is capable of detecting and responding to a variety of sounds produced outside of its mother. Expectant mothers report fetal movements in response to intense sounds, e.g, barking dogs, weapons discharge, and rhythmic, low-frequency drum beats. Motor responses are dependent on a number of factors including the characteristics of the stimulus (waveform, level and frequency) and the sleep state of the fetus (Kisilevsky et al., 1989; Lecanuet et al., 1992; Lecanuet and Schaal, 1996). Quantitative evidence of changes in both electrocortical activity and heart-rate responses supports the common subjective reports of fetal movements.

The fetus is not completely isolated from sounds in the mother's environment. Depending upon the characteristics of the stimulus, a significant quantity of the signal is present at the fetal head. Steady-state noises are filtered by tissue and fluids before reaching the fetal head (Peters et al., 1991; Gerhardt KJ, et al., 1990). Low-frequency sounds, below about 400 Hz, are transmitted into the uterus will little loss in sound pressure, whereas higher frequency sounds (from 400- 4000 Hz) are reduced by about 6 dB/octave (Gerhardt et al., 1990). Sound energy at the fetal head reaches the fetal inner ear via bone conduction (Gerhardt et al., 1992). This pathway to the fetal inner ear introduces additional signal reduction, particularly in the frequency region greater than about 500 Hz.

Impulse noise forms a different class of sounds than steady-state noise. Impulses include all forms of high-intensity, short-duration sounds, for example, those from impacts produced by the collision of two objects to those intense impulses associated with the discharge of a weapon. The level of an impulse recorded with a hydrophone in the uterus of a pregnant sheep is highly dependent upon the distance of the recording device to the internal abdominal wall (Nijhuis and van de Pas, 1992). However, the rate of attenuation by frequency for impulse noise is similar to that of steady-state noise (Nijhuis and van de Pas, 1992).

Steady-state vibroacoustic stimulation, produced by a mechanically vibrating sound source applied directly to the abdomen, induced changes in the behavioral state of human fetuses (Nijhuis and van de Pas, 1992) and sheep fetuses (Bauer et al., 1997; Abrams et al., 1996). The use of sheep as an animal model for human fetal response to sound stimulation has been used recently

because sheep fetuses hear before birth (Woods, et al., 1984), as do human fetuses (Birnholz and Benacerraf, 1983) and because of dimensional and, therefore, sound transmission similarities of the abdomen (Peters et al., 1991; 1993).

Steady-state vibroacoustic stimulation applied to the abdomen of pregnant sheep, while the fetus is in either rapid eye movement (REM) or non-rapid eye movement (NREM) sleep, induced marked changes in the electrocortical activity of the fetus (Bauer et al., 1997; Abrams et al., 1996). Whether or not impulse noise evokes similar responses in fetal sheep has not been determined. The present experiment was designed to answer this question.

Six ewes, all carrying singlet fetuses between 126 and 129 days gestational age (term = 145 days), were anesthetized with 2% halothane in oxygen. Animals underwent a midline abdominal incision and hysterotomy using standard, aseptic techniques. All procedures involving animals were approved by the University of Florida's Committee for the Care and Use of Animals.

The fetal head was delivered and the scalp was incised along the midline with a right angle flap extending over both superior orbital ridges. Two stainless steel screw electrodes (0-80) with electrode wires attached were placed on the dura mater over the parietal cortex for recording the electrocorticogram (ECoG). Two electrodes for recording the electrooculogram (EOG) were placed into each orbit through pre-drilled holes in the superior orbital ridge. Thin layers of methyl methacrylate were placed over the screws to stabilize and insulate all electrodes. The scalp was closed with silk thread. To record the electrocardiogram (ECG), stainless steel wire electrodes (Cooner Wire, Inc., Chatsworth, CA) were sutured to subdermal tissues above the sternum and over a spinal process of a thoracic vertebra. To measure the amplitude of intrauterine impulse sound pressure, and its subsequent effect on instantaneous heart rate and electrocortical activity, a miniature hydrophone was attached to the skin overlying the fetal temporal bone.

Sodium ampicillin, 500 mg, was placed in the uterine and peritoneal cavities before closing these incisions. Electrode wires and the hydrophone cable were tunneled to a location high on the flank of the ewe where they were brought out through a small incision and stored in a pouch sutured to the skin. Ewes were returned to their pens and received penicillin and streptomycin each day for three days following surgery.

On the day of the experiment, 3-5 days post-op, ewes were brought into the laboratory, placed in an elevated, custom-built pen, which was open on the sides to expose the flank of the ewe, and provided with water and food. Fetal ECoG, EOG, and ECG signals were amplified and filtered (band-pass ranges: ECoG and EOG, 0.3-30 Hz; ECG 30-300 Hz), and recorded continuously on a physiograph (model 24005, Gould Instruments, Dayton, OH) and FM tape (model 7005 Bruel and Kjaer Instruments, Inc. Marlborough, MA). The hydrophone was amplified (B&K Charger Amplifier type 2635) and its output was delivered to a Frequency Analyzer (B&K Type 2133) and also recorded simultaneously on the polygraph for exact stimulus detection.

Each of the six ewes was exposed to 20 impulses delivered during a 30-minute period of time. A shock tube was used to produce air-borne impulses (duration of <1 ms) directed to the pregnant sheep's flank. There was a distance between the open end of shock tube and abdominal wall of 1 meter. Animals were stimulated in epochs of NREM and REM sleep not earlier than two minutes after a clear sleep-state change was visible on a slow moving strip chart (0.25mm/sec). Quiet sleep, or NREM sleep, was characterized by occasional muscular tone and activity, a high-voltage, low-frequency ECoG pattern, and absence of sustained, rapid eye

movements. Periods during which these criteria could not be applied were termed Indeterminate (Szeto and Hinman, 1985). The assessment of behavioral state was made off-line by visual inspection of the strip-chart recordings of ECoG, EOG and EMG. Two investigators blinded to the experimental protocol made this assessment.

Quantification of the fetal ECoG was also performed using Fast Fourier Transformation (FFT). Data were stored on FM tape and were fed into a PC using a 16-channel A/D board (Data Translation, DT2821F, Marlboro, MA). Sample rates of 128 Hz were used to record ECoG and eye movements, 2048 Hz to record ECG, and 256 Hz to record neck EMG. Quantification of the fetal ECoG was performed using Fast Fourier Transformation (FFT). The assessment of fetal behavioral state was confirmed on computer screen (using the software package ATISA FOR WINDOWS®, Schwind Elektronik, Erlangen, Germany).

Signals were quantified continuously over sixty-second periods beginning 20 seconds prior to impulse stimulation in both REM and non-REM behavioral states. For heart rate calculations, the individual R waves, with the R wave peak as the trigger point, were sequentially recognized. Correct R wave peak detection was checked and corrected individually by continuous play back so that an artifact-free heart rate processing resulted. The distance between consecutive R wave peaks were measured with a precision of 0.48 ms. The series of R-R intervals (T1, T2, T3, ..., Tn) was stored as a function of the beat number. This series constitutes the tachogram. The reciprocal of this series represented the instantaneous fetal heart rate (FHR in bpm).

Dynamic ECoG analysis was performed by discrete Hilbert transformation which was realized by fast Fourier transform for different spectral bands (total band: 1.5-30 Hz; Delta band: 1.5-4 Hz; Theta band: 4-8 Hz; Alpha band: 8-13 Hz; Beta band: 13-30 Hz) (Witte et al., 1991). Therefore, momentary spectral band powers were calculated and normalized by division of summarized spectral band power of the time intervals analyzed. Furthermore, band powers in different frequency bands were analyzed for NREM sleep and REM sleep states during seven different periods (20 to 10 seconds, 10 to 5 seconds, and 5 to 0 seconds before impulse stimulation and 2 to 5 seconds, 5 to 10 seconds, 10 to 20 seconds, and 20 to 60 seconds after stimulation). Spectral band power was normalized with the prestimulation spectral band power for the –5 to 0 seconds period. The period from 0 to 2 seconds was excluded because of possible interference with short but intensive EOG fluctuations that almost always occurred immediately after impulse stimulation.

Derived ECoG and FHR parameters were separately averaged for each behavioral state and each animal, and the respective means were used for the statistical analysis. Data were reported as means ± SD. An analysis of variance (ANOVA) for repeated measures was applied to the data and was followed by the Dunnett's test. The purpose of the latter analysis was to compare the means of parameters obtained during baseline conditions (the period -5 to 0 seconds before impulse noise stimulation) with the other periods 20 seconds before, during and 40 seconds after stimulation. The alpha level was set at 0.05.

Altogether, 117 responses to impulse noise stimulation were studied in six near-term fetal sheep. Impulses occurred during NREM sleep (n=59), REM sleep (n=45), and Indeterminate state (n=13).

A shock tube produced impulses that averaged 169.4 dB (S.D. 0.92) pSPL in air. Intrauterine recorded impulse levels were quite similar between the animals studied and also

during the individual series. Peak levels recorded near the fetal head averaged 166.1 dB (S.D. 5.94).

Exposure to impulses resulted in significant changes in heart-rate dynamics and the spectral content of cortical activity during both REM and NREM sleep. However, behavioral state changes were not detected as assessed from gross observation of the polygram strip chart set on a slow speed (Figure 21).

Impulses delivered to the fetus during periods of NREM sleep resulted in a decrease in an average heart-rate from 185 ± 22 bpm before stimulation to 174 ± 23 bpm 2 to 5 seconds after stimulation ($p < 0.05$; Figure 22, left panel). Changes in heart rate were normalized to 100% with respect to the average heart rate during the period from -5 to 0 seconds before stimulation. Heart rate variability as judged from the standard deviations in Figure 22 was greater during and up to 20 sec after stimulation than before stimulation.

Figure 23 shows the effects of impulse noise on the frequency content of the ECoG before and after exposure. Spectral power for each time-period was referenced to the –5 to 0 second period prior to stimulation (referenced as 100%). The most consistent change occurred in the Delta-band. Impulse exposure during NREM sleep resulted in a reduction in Delta-band power from 100% to 63%± 18%. A similar decrease was found in Theta-band power (from 100% to 66% ± 21%) and in Alpha-band power (from 100% to 70% ± 17%). Consequently, total power decreased from 100% to 72% ± 16% ($p < 0.05$). In contrast, spectral power in the Beta-band was not altered. Immediately after stimulation, the ratio between spectral power in the higher and lower bands was significantly increased ($p < 0.05$) (Figure 23).

During REM sleep, impulse stimulation led to a small but systematic increase in fetal heart rate from 177 ± 24 bpm to 189 ± 31 bpm ($p < 0.05$; Figure 22, right panel). Heart rate variability increased during and up to 5 sec after stimulation.

During REM sleep, stimulation provoked a short decrease in total-band power from 100% to 73% ± 20% and a similar decrease in the Theta-band power (from 100% to 75% ± 13%) and Beta-band (from 100% to 80% ± 10%) (Figure 24). Spectral power in the Delta- and Alpha-bands, the ratio between higher and lower band powers, was not altered. Figure 24 indicated that when changes did occur from baseline values, they quickly returned to normal shortly after stimulation.

The most frequently occurring types of sounds that cause fetal movements are those of an episodic nature. Pregnant women at term often find movements to be vigorous and sufficiently distracting to seek quieter places. Apart from these generalized motor responses, the specific and subtle effects of impulse sounds on fetal behavioral state have not been clearly elucidated. The pregnant ewe was felt to be a good animal model because of its extensive use in the past for evaluation of the effects of continuous sound on fetal heart rate ECoG and EOG (Abrams et al., 1996), transmission properties of speech (Griffiths et al., 1994) and music (Abrams et al., 1998), and increased glucose utilization along the central auditory pathway in sheep fetuses (Abrams et al., 1998).

Along with the well-recognized physiologic responses of the fetus to intense sound, it is now known that high-intensity impulses produce damage to the fetal inner ear. In a study by Gerhardt et al. (1998), eleven pregnant ewes carrying fetuses at gestational ages of 127 days were exposed to impulses with a peak level of 169.7 dB. Slight elevations of fetal auditory evoked potentials were noted for low-frequency stimuli. Scanning electron microscopy revealed damage

to hair cells in the middle and apical turns of the cochlea. The levels used in the current study were similar to those levels reported by Gerhardt et al.

Table 5 summarizes the results from the current study as well as similar studies in fetal sheep that used different types of stimulation. Impulse stimulation during REM and NREM sleep produced consistent changes in the FHR; during REM sleep FHR increases and during NREM FHR decreased. Bauer et al. (1997) found a similar increase during REM sleep and a decrease during NREM in response to stimulation with an electronic artificial larynx placed directly on the abdomen.

Table 5. Summary of the results from the current study compared to previous studies. Differences in fetal heart rate (FHR) and electrocorticograms (ECoG) between pre-stimulation and during or after stimulation are indicated as: NC= no change; I= increase above pre-stimulation; D= decrease below pre-stimulation; -- = not studied. Gross changes in slow speed strip-chart readings of behavioral state are indicated as: NC= no change; and DISR= disruption, a change to another sleep state.

		Current Study	Bauer et al.	Abrams et al.
	Stimulus	***Impulse***	***EAL***	***VAS***
Sleep State		**REM**	**REM**	**REM**
FHR		I	I	--
Strip Chart		NC	NC	NC
ECoG	Delta	NC	NC	NC
	Theta	D	D	D
	Alpha	NC	I	NC
	Beta	D	I	I
	Total power	D	NC	NC
Sleep State		**NREM**	**NREM**	**NREM**
FHR		D	D	--
Strip Chart		NC	NC	DISR
ECoG	Delta	D	D	D
	Theta	D	D	D
	Alpha	D	NC	NC
	Beta	NC	NC	NC
	Total power	D	D	D

Changes in FHR evoked by acoustic stimulation may reflect a combination of respiratory influences that are vagally mediated (Davidson et al., 1992; Groome et al., 1994) and state influences that are primarily sympathetically mediated (Davidson et al., 1992). In the present study, fetal heart rates were significantly different from pre-exposure heart rates for 2-5 seconds. Longer heart rate variations, up to one hour, have been reported in human fetuses following EAL stimulation (Visser et al., 1989). Heart rate changes of this magnitude were not considered to be

physiologic but rather may have reflected "pain". Impulse stimulation did not result in this type of fetal response in sheep. However, it should be noted that the response of fetal sheep is blunted compared to that of human fetuses.

In the current study, changes in brain activity following impulse exposure were noted during both REM and NREM sleep. Impulse noise resulted in a *decrease* in amplitude of the ECoG in most frequency bands regardless of sleep state (Table 5). A decrease in the low-frequency component of the ECoG (Delta and Theta) is indicative of a desynchronization of rhythmic cortical high-voltage activity (Steriade et al., 1990) and is mediated by the diffuse thalamocortical projections of the reticular formation (Ingvar and Söderberg, 1958; Meyer et al., 1969). A specific arousal-like pattern, characterized by an *increase* in amplitude of the high-frequency components of the ECoG (EEG, 1992), did not occur following impulse stimulation.

In contrast, previous studies that used viboacoustic stimulation during REM sleep showed an *increase* in the high-frequency components of brain activity (see Table 5). Thus, the impulse stimulus had a different effect on the fetus than did the vibroacoustic stimulus. Characteristics of the stimulus, including its amplitude, duration, onset-offset patterns and spectral information were probably responsible for producing these different response patterns.

In fetal sheep, vibroacoustic as well as impulse stimulation evoked an arousal response (changes in the band powers of the ECoG) without a concomitant interruption of the behavioral state as evidenced by inspection of strip-chart recordings of ECoG, EOG and EMG. A stimulus-related disruption of the synchronized rhythmic slow-wave activity of the ECoG is largely generated through a cyclical interaction between thalamocortical and thalamic reticular neurons (McCormick and Bal, 1997). Thus, stimulation of high-frequency brain activity probably requires a different mechanism in fetal sheep than in humans.

The quantification of the spectral components of the fetal electrocorticogram permitted extraction of clear central nervous system responses to impulse sounds. Failure to detect these subtle changes from slow moving strip-chart recordings does not diminish the importance of the fetal response.

In adult cats, electrical stimulation of the brain stem resulted in the elimination of the cortical slow wave rhythm that occurred with a latency of 1.2 sec (Steriade et al., 1993). In the current experiments, the rapid onset of cortical desynchronization (2 to 5 sec) after impulse stimulation (Figure 23, NREM, Delta band) indicated that brainstem-thalamocortical connections were well-developed in near-term fetal sheep. The *timing* of the changes in the frequency content of the ECoG that occur after impulse stimulation as well as the *direction* of amplitude changes in the two behavioral states as presented in Table 5 agree with those of previous studies (Bauer et al., 1997; Abrams et al., 1996).

In summary, impulses at levels capable of producing damage to the hair cells of the cochlea, as previously reported (Gerhardt et al., 1998), can also evoke consistent alterations in FHR and ECoG patterns. These patterns are characterized by a stimulus-related ECoG desynchronization without interruption of the behavioral state. Whether or not the effects of impulse stimulation on behavioral state and heart rate response have any long-term consequences as they appear to have on inner ear hair cells of the fetus, and perhaps even the newborn lamb, remains to be established.

Recommendations Related to Statement of Work

Below is a summary of the tasks listed in the Statement of Work:

Tasks Proposed:	Status:
Order ABR and AMFR recording equipment	Completed
Construct shock-tube	Completed
Test shock tube (air measurements)	Completed
Confirm shock tube signature in water	Completed
Collect data on ten animals (Study 1)	Completed
Submit annual Report	Completed
Collect data on 20 animals (Study 2)	Completed
Evaluate histopathology	Completed
Final Report to U.S. Army	Completed

One manuscript is in press, a second is under review and a third is nearly ready for submission.

CONCLUSIONS

Peak SPLs recorded from within the uterus were highly variable and ranged from 153 to 168 dB. Peak levels in air averaged 169.9 dB. The overall morphology of the waveforms related to pSPL and frequency content of the impulse. Peak levels recorded in the uterus averaged 7.3 dB less than those recorded in air. Spectral analysis in one-third octave-bands revealed peak levels in air at 315 Hz compared to 160 Hz when recorded from the uterus. High-frequency sound pressures were attenuated by the tissues and fluids of the ewe by up to 25 dB, as predicted from earlier studies.

The position of the hydrophone within the uterus influenced both pSPL as well as spectral distribution. When the hydrophone was near the abdominal surface, peak levels were approximately 2 dB less than the peak levels recorded in air. When the hydrophone was deep within the uterus, the morphology of the waveform changed and peak levels were approximately 20 dB less than those recorded in air.

Electrophysiologic thresholds were examined over time. Small elevations in the mean thresholds for the 0.5 kHz stimuli (ABR) were noted in the post-exposure measures. Thresholds improved in recordings over the next 10 days. No similar elevations were noted for the higher-frequency tone bursts or clicks, or for the AMFR. Similarly, continuous, intense broadband noise exposures delivered to fetal sheep resulted in temporary post-exposure threshold elevations for low-frequency tone bursts (Griffiths et al., 1994). ABR latencies shortened as gestational age increased in the early noised-exposed fetuses. Exposure to impulses did not affect ABR latencies in either group of fetuses.

Cochleae from the fetuses were examined using scanning electron microscopy. Hair cells from noise-exposed fetuses appeared different in a number of respects from historical control fetuses from this laboratory (Gerhardt et al., 1999). Damage to both inner and outer hair cells was noted primarily in the apical and middle turns of the cochlea. Abnormalities included bent and/or missing stereocilia, giant stereocilia and phalangeal scarring. The damage found in fetal sheep inner ears is consistent with reports following noise exposures in other species (Saunders et

al., 1985). Inner hair cells were more severely affected than outer hair cells in the apical region between 5 and 20% of the total distance from helicotrema. These findings are quite different than would be predicted based upon knowledge of adult noise-induced hearing loss that affects the basal region of the cochlea.

The findings that noise exposure to the fetus *in utero* affect inner ear histology apply only to fetal sheep. There are no compelling data to demonstrate that human fetuses have the same susceptibility to noise as do sheep, or that human fetuses are at risk to inner ear damage produced by noise levels to which pregnant women might normally be exposed. However, the data from this study warrant consideration in the formulation of guidelines that may be developed to protect the fetus of pregnant women from noise damage.

Impulses delivered to the fetus during periods of NREM sleep resulted in a decrease in average fetal heart rate (FHR) from 185 ± 22 beats/minute (bpm) before stimulation to 174 ± 23 bpm 2 to 5 seconds after stimulation ($p < 0.05$). During REM sleep, a FHR acceleration occurred (before stimulation: 177 ± 24 bpm; after stimulation: 189 ± 31 bpm; $p < 0.05$). Impulse exposure during NREM sleep resulted in reductions in Delta-, Theta- and Alpha-band powers. Consequently, total power decreased from 100% to 72% ± 16% ($p < 0.05$). During REM sleep, stimulation provoked a short decrease in total-band power from 100% to 73% ± 20% and a similar decrease in the Theta- and Beta-band powers. The results indicated that impulse noise evoked short-term alterations in fetal heart rate and cortical activity. These changes were mediated by auditory brain stem activation that led to cortical desynchronization during both NREM and REM sleep in late-term fetal sheep.

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APPENDIX

Figures 1 through 24

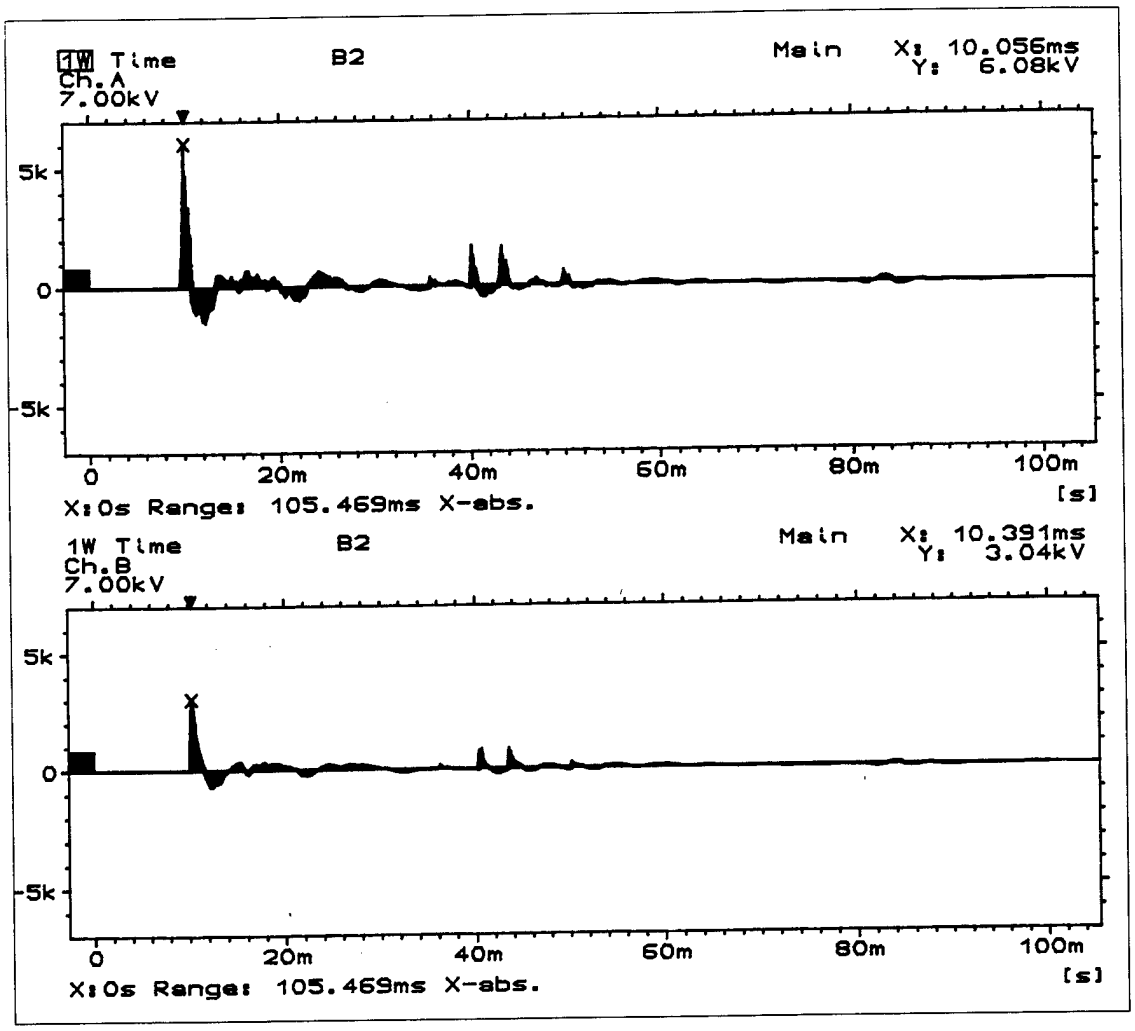


Figure 1. Waveforms of an impulse recorded simultaneously with hydrophones in air (upper panel) and in the abdomen (lower panel) of non-pregnant sheep. The intraabdominal hydrophone was within 1 inch of the flank nearest the shock tube (proximal). The pSPLs were 170 and 167 dB in air and in the abdomen, respectively.

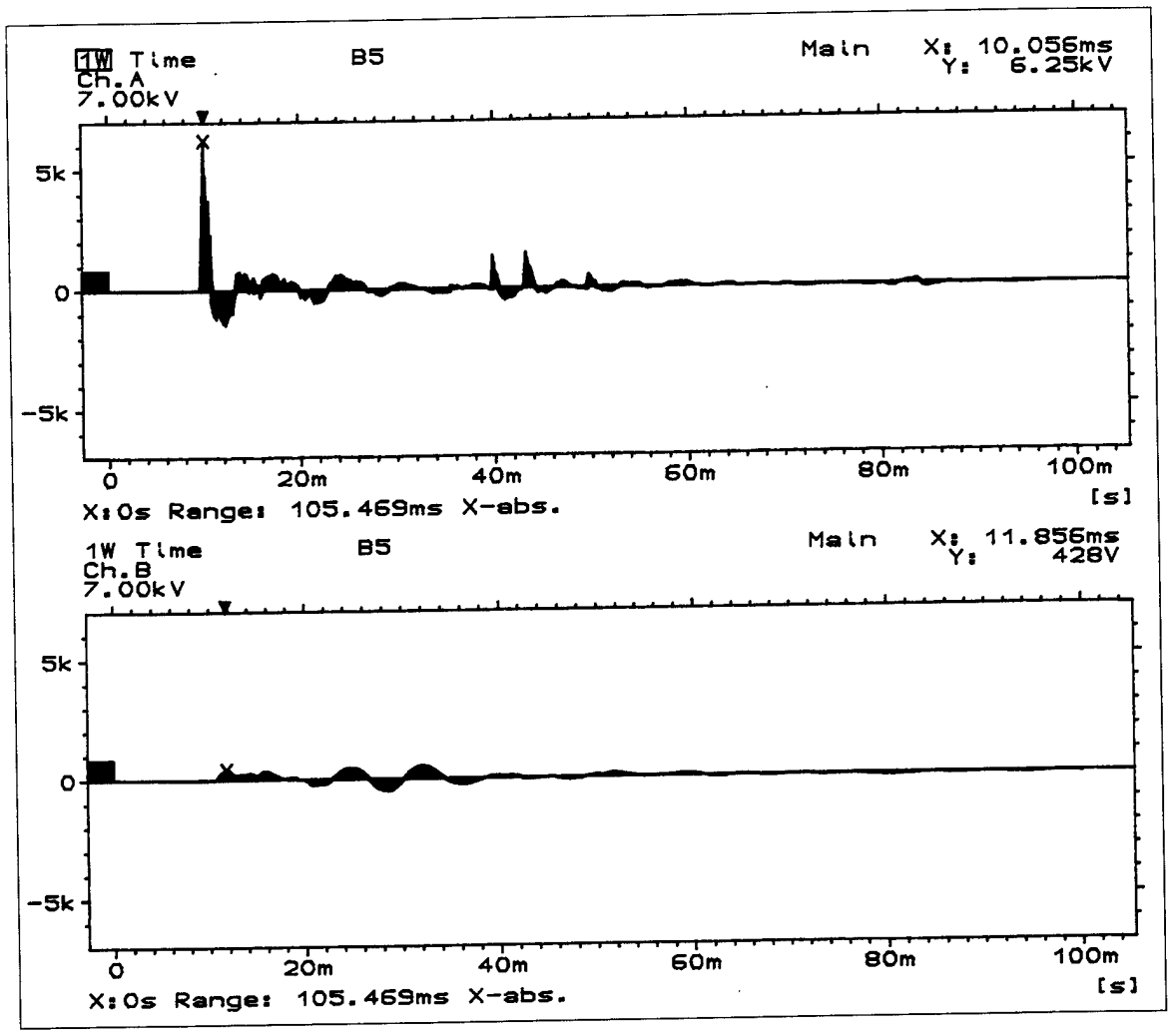


Figure 2. Waveforms of an impulse recorded simultaneously with hydrophones in air (upper panel) and in the abdomen (lower panel) of non-pregnant sheep. The hydrophone was deep within the abdomen at midline (medial). The pSPLs were 170 and 147 dB in air and in the abdomen, respectively.

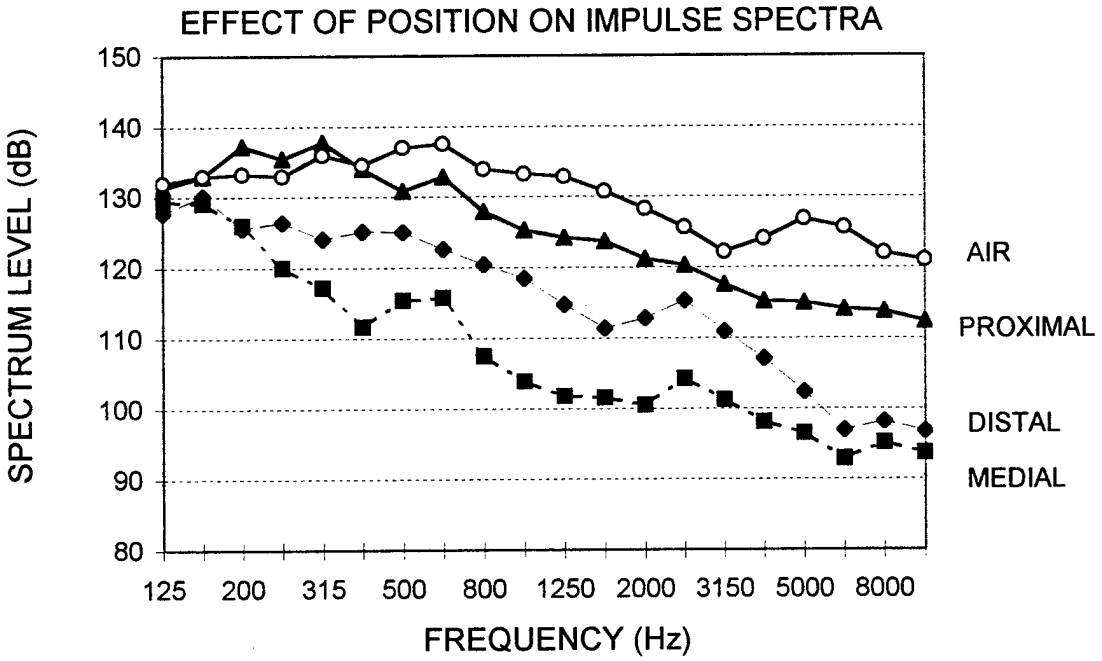


Figure 3. Spectra of impulses recorded with hydrophones in air and positioned at three locations within the abdomen of non-pregnant sheep. Proximal - the hydrophone was within one inch of the surface of the flank closest to the shock tube. Medial - the hydrophone was positioned at the midline of the abdomen. Distal - the hydrophone was within one inch of the surface of the flank farthest from the shock tube.

Figure 4. Electrophysiologic Thresholds in the Early-Exposed Group

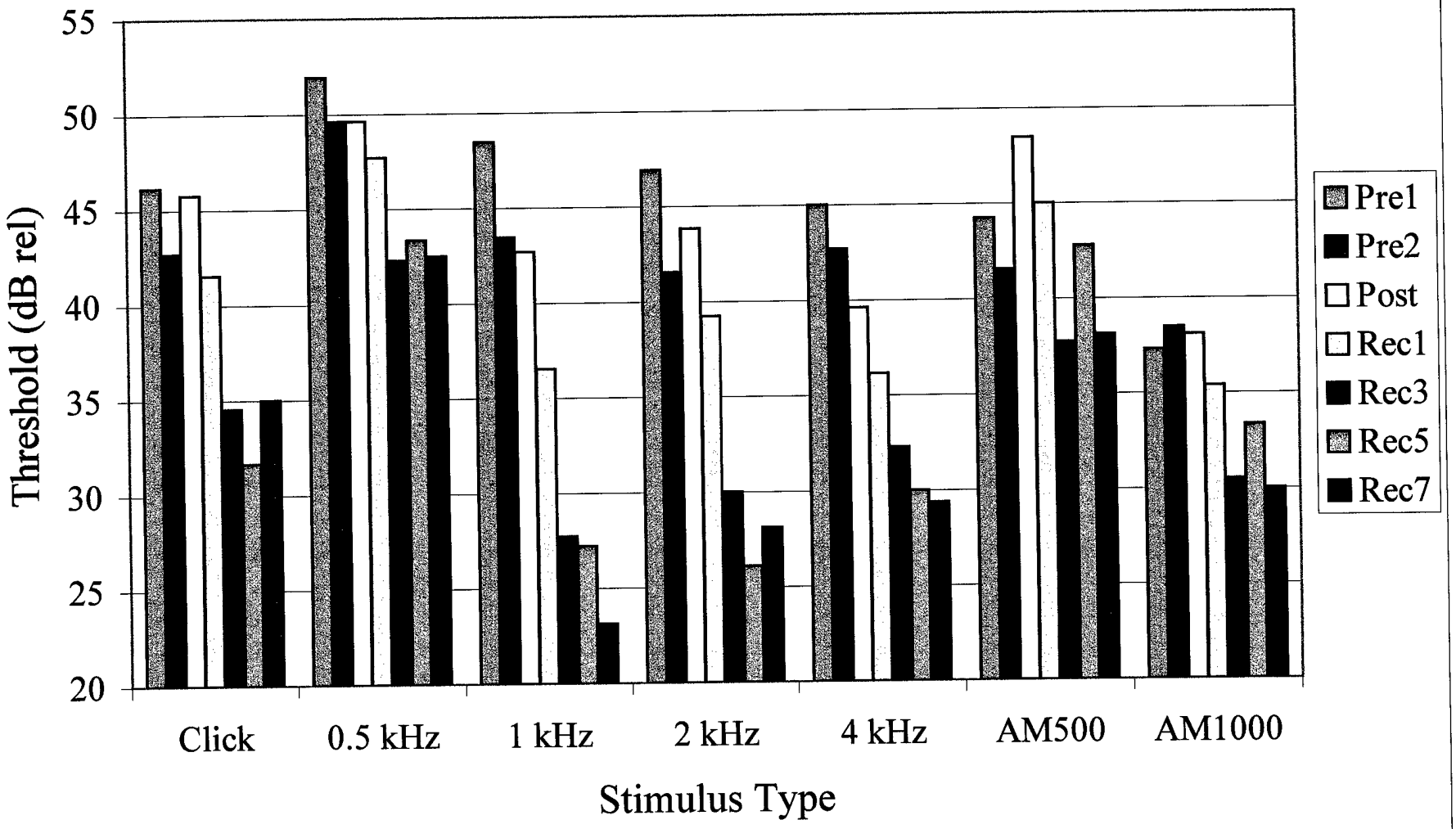


Figure 5. Electrophysiologic Thresholds in the Late-Exposed Group

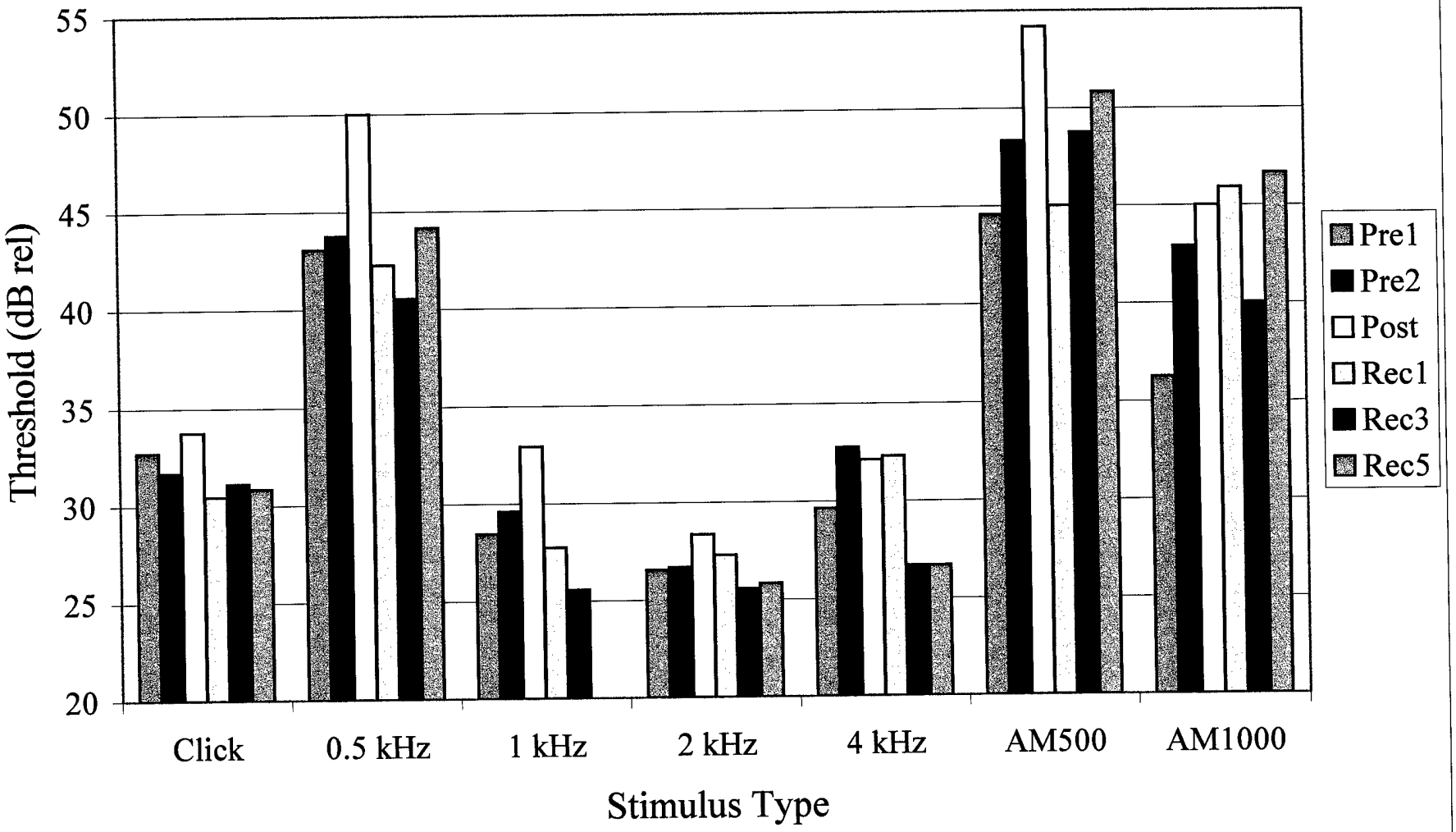


Figure 6. Click-Evoked ABR Wave IV Latency Shifts (Late-Exposed)

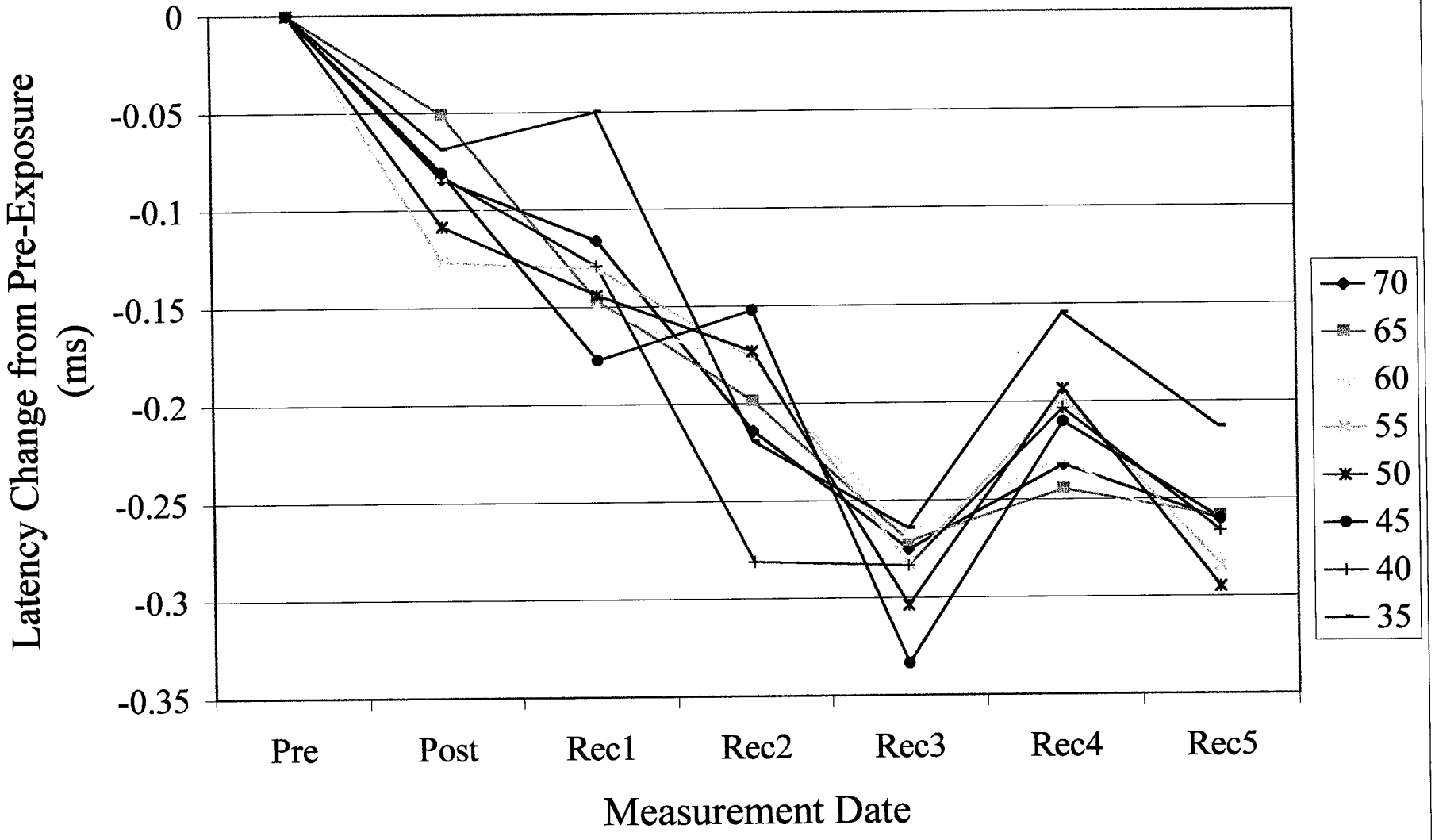


Figure 7: 4 kHz ABR Wave IV Latency Shifts (Late-Exposed)

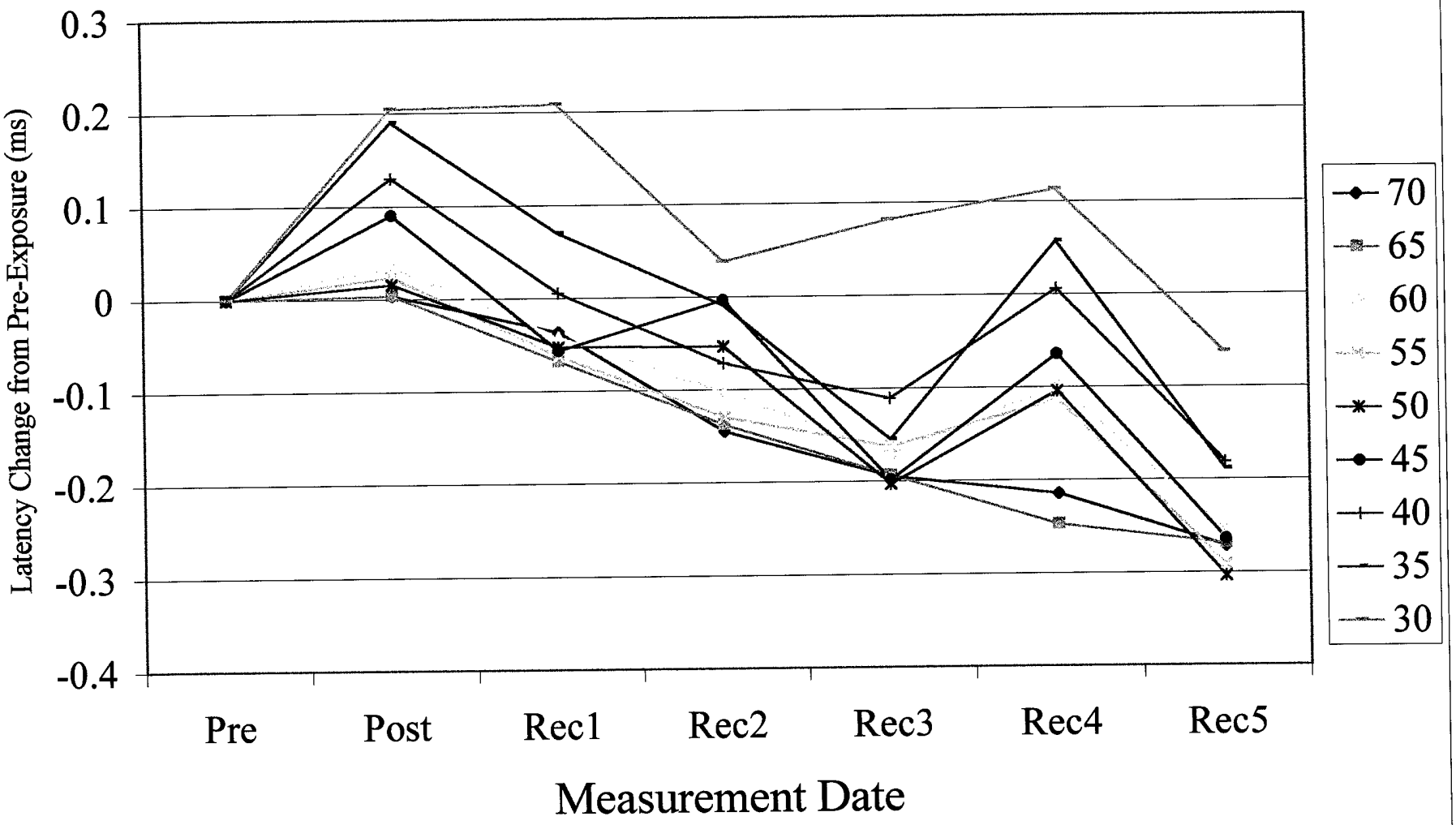


Figure 8. 2 kHz ABR Wave IV Latency Shifts (Late-Exposed)

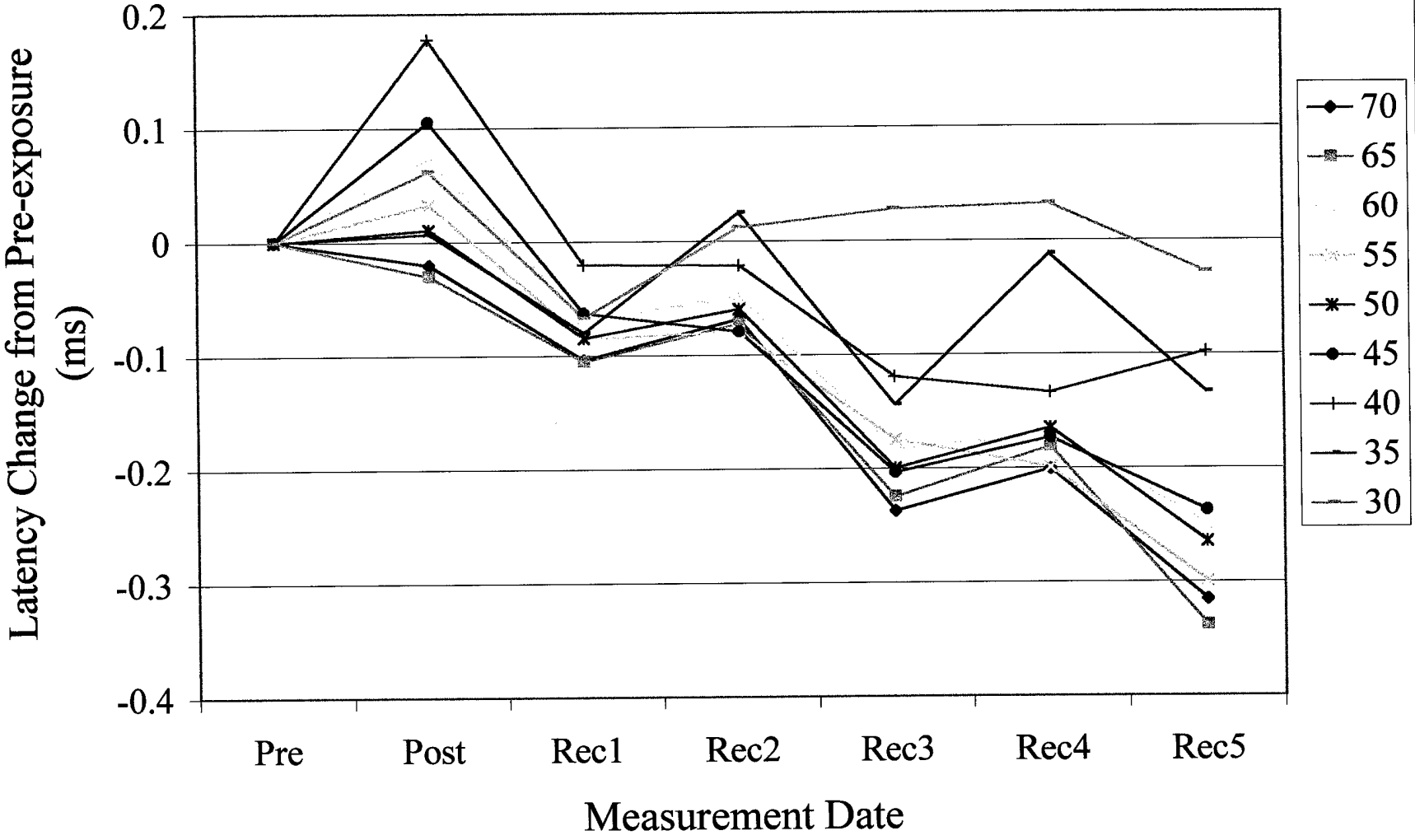


Figure 9. 1 kHz ABR Wave IV Latency Shifts (Late-Exposed)

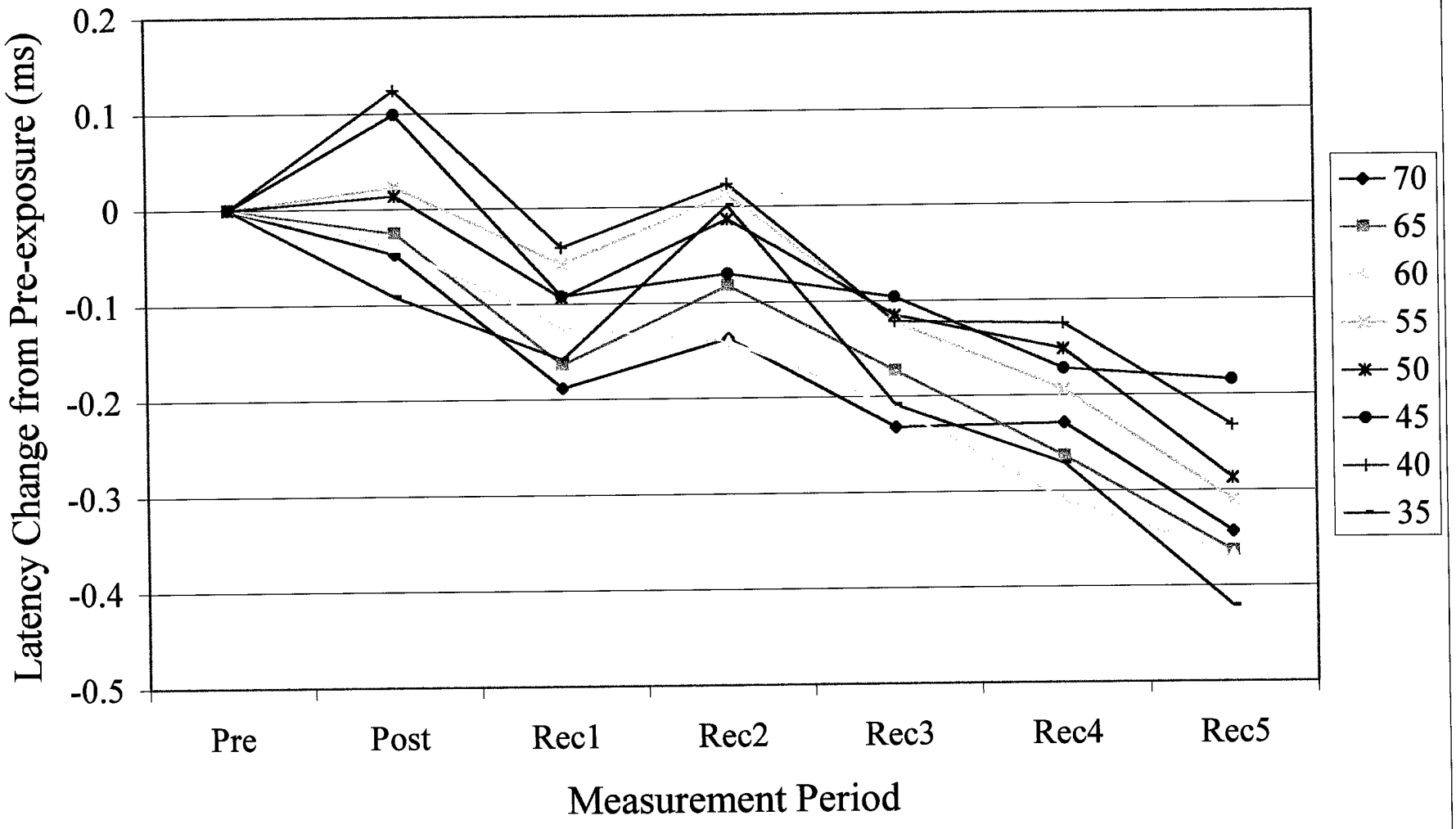


Figure 10. Click-evoked ABR Wave IV Latency Shifts (Early-Exposed)

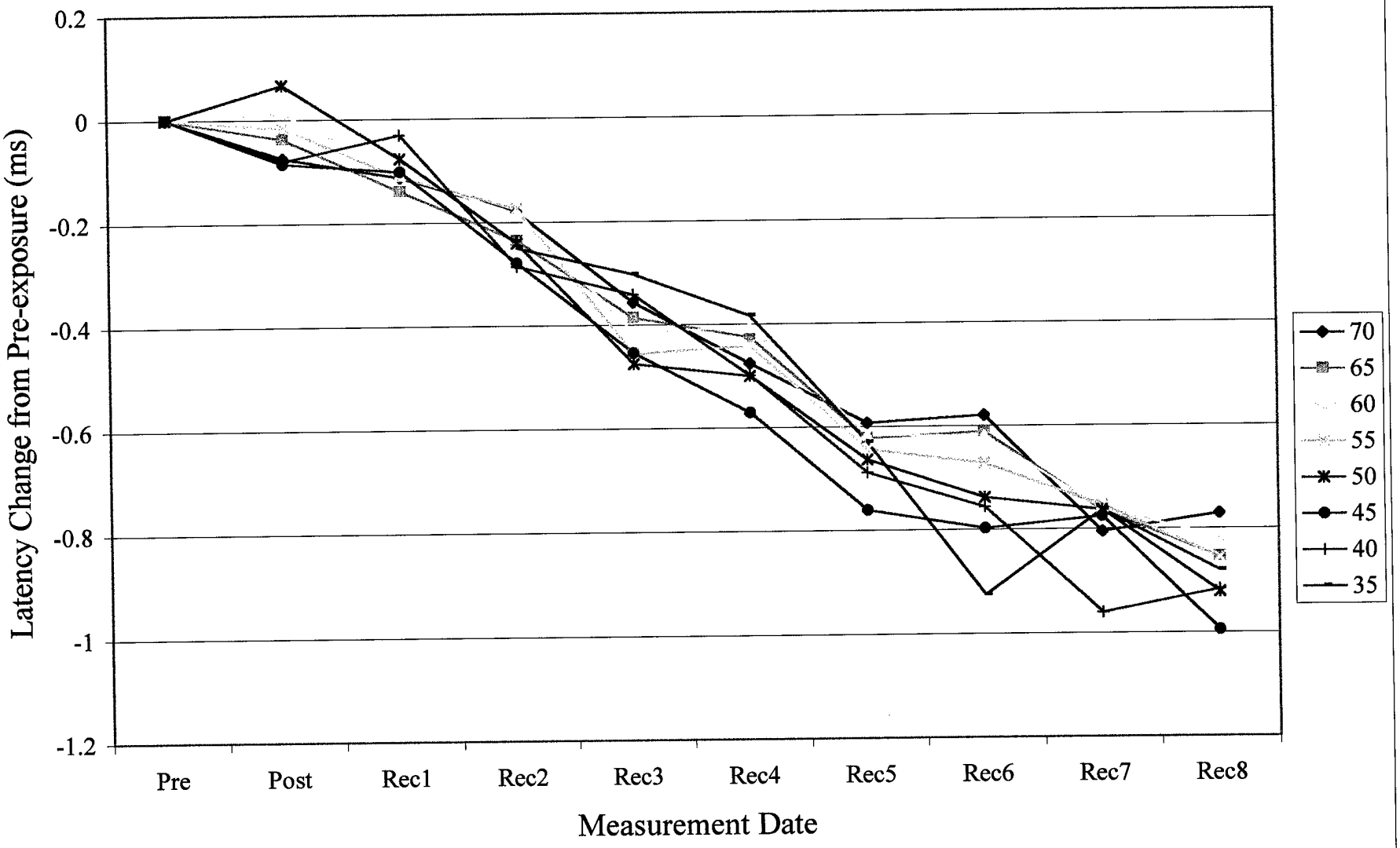


Figure 11. 4 kHz ABR Wave IV Latency Shifts (Early-Exposed)

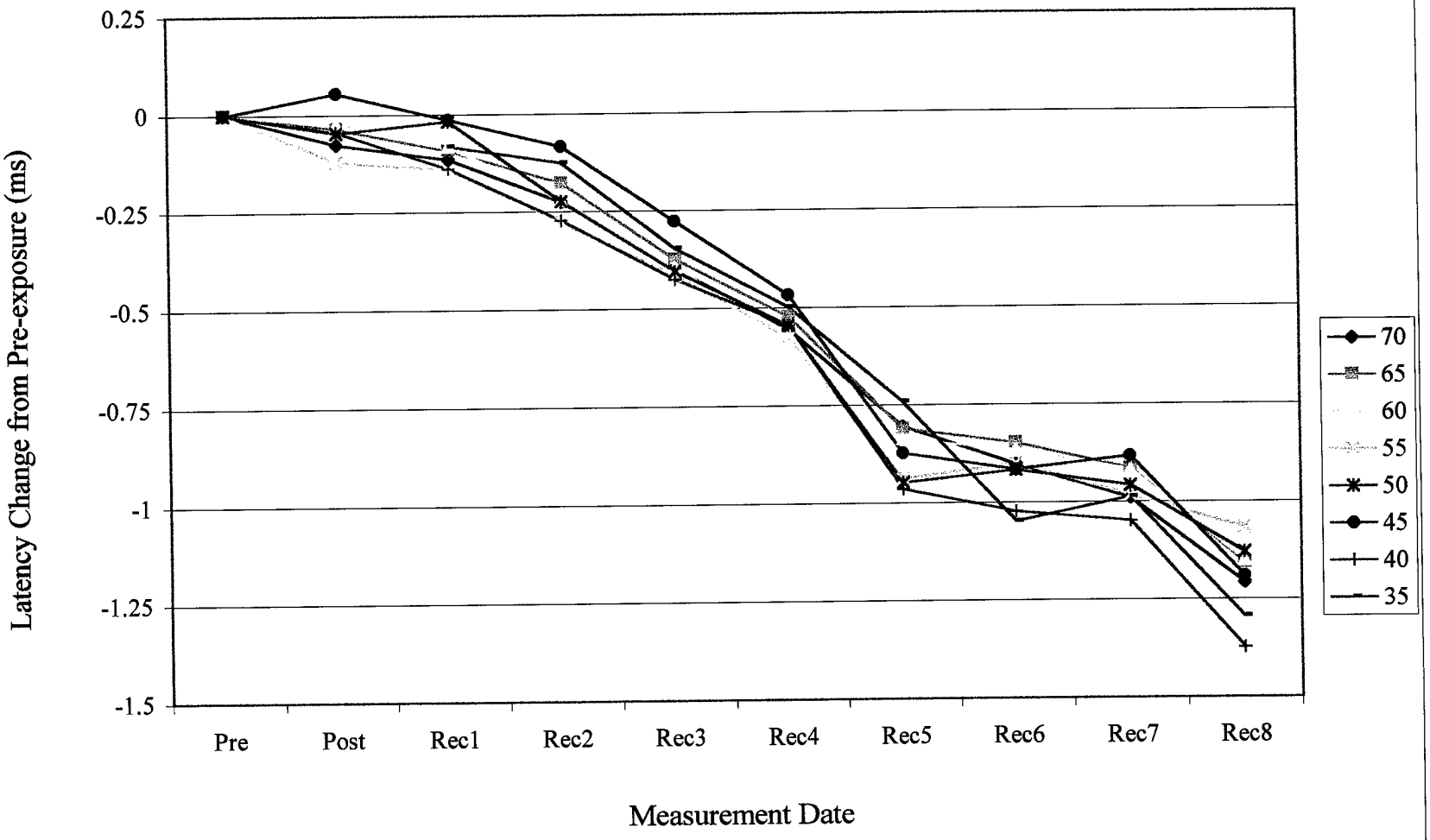


Figure 12. 2 kHz ABR Wave IV Latency Shifts (Early-Exposed)

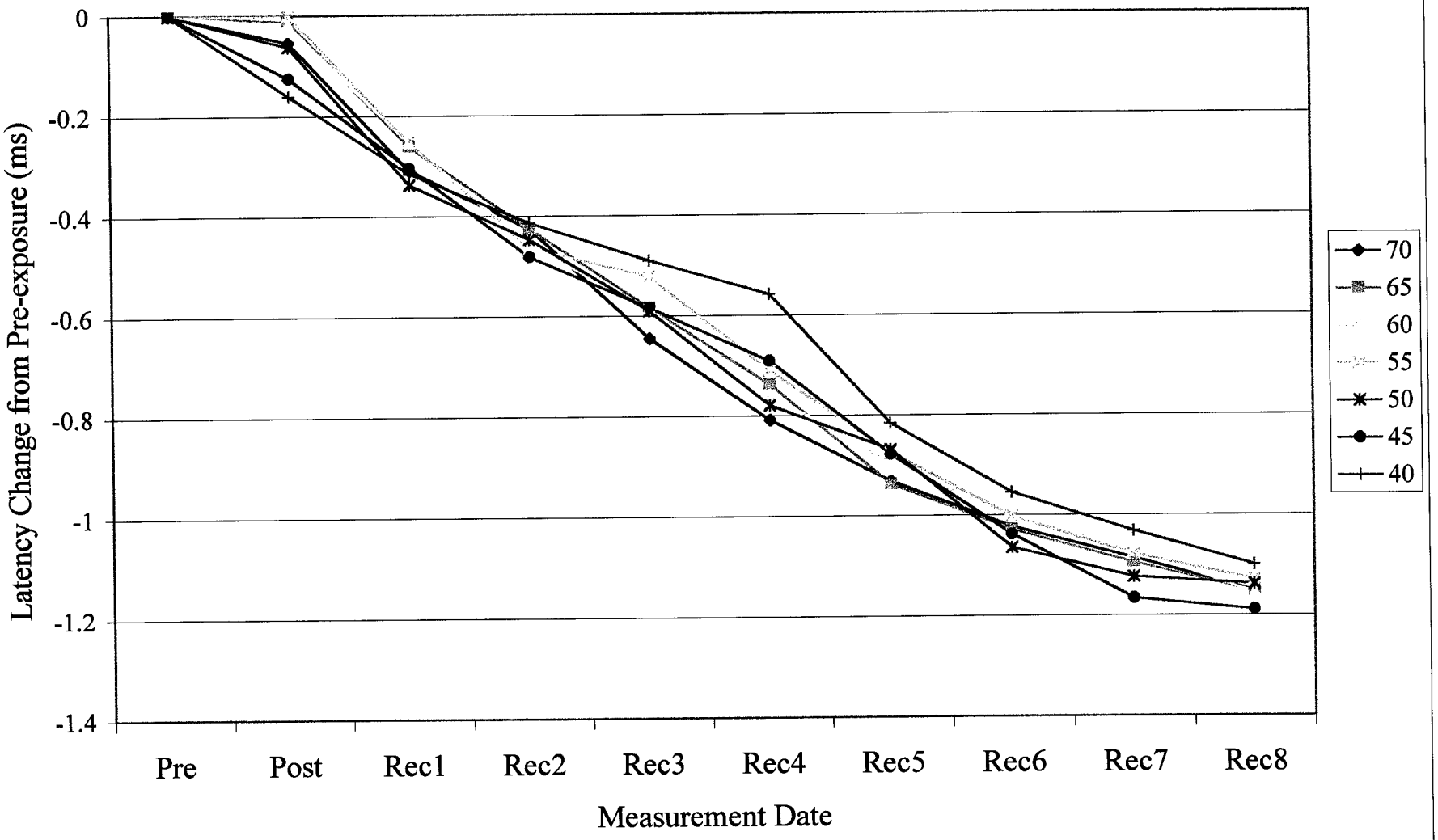


Figure 13. 1 kHz ABR Wave IV Latency Shifts (Early-Exposed)

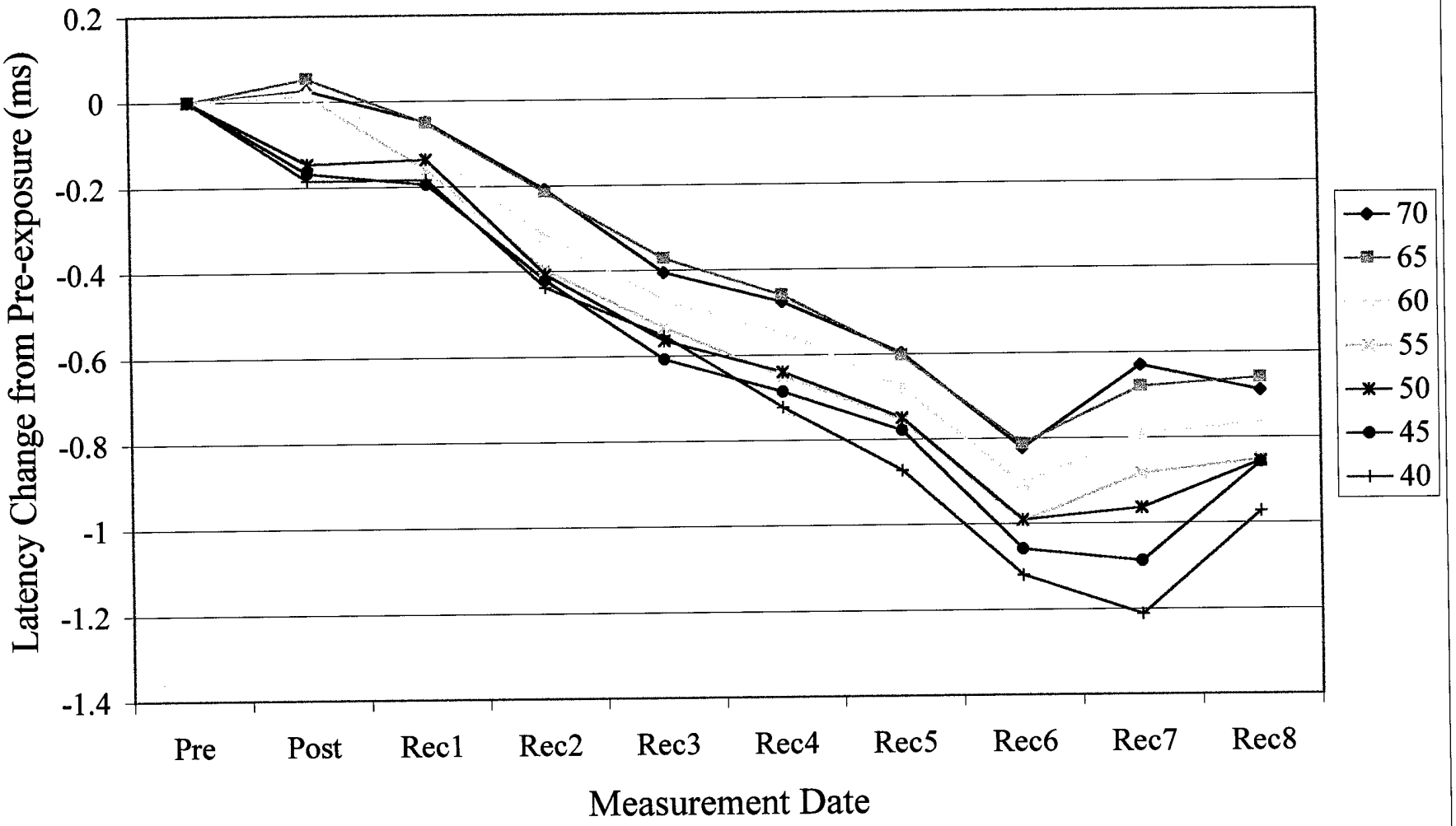
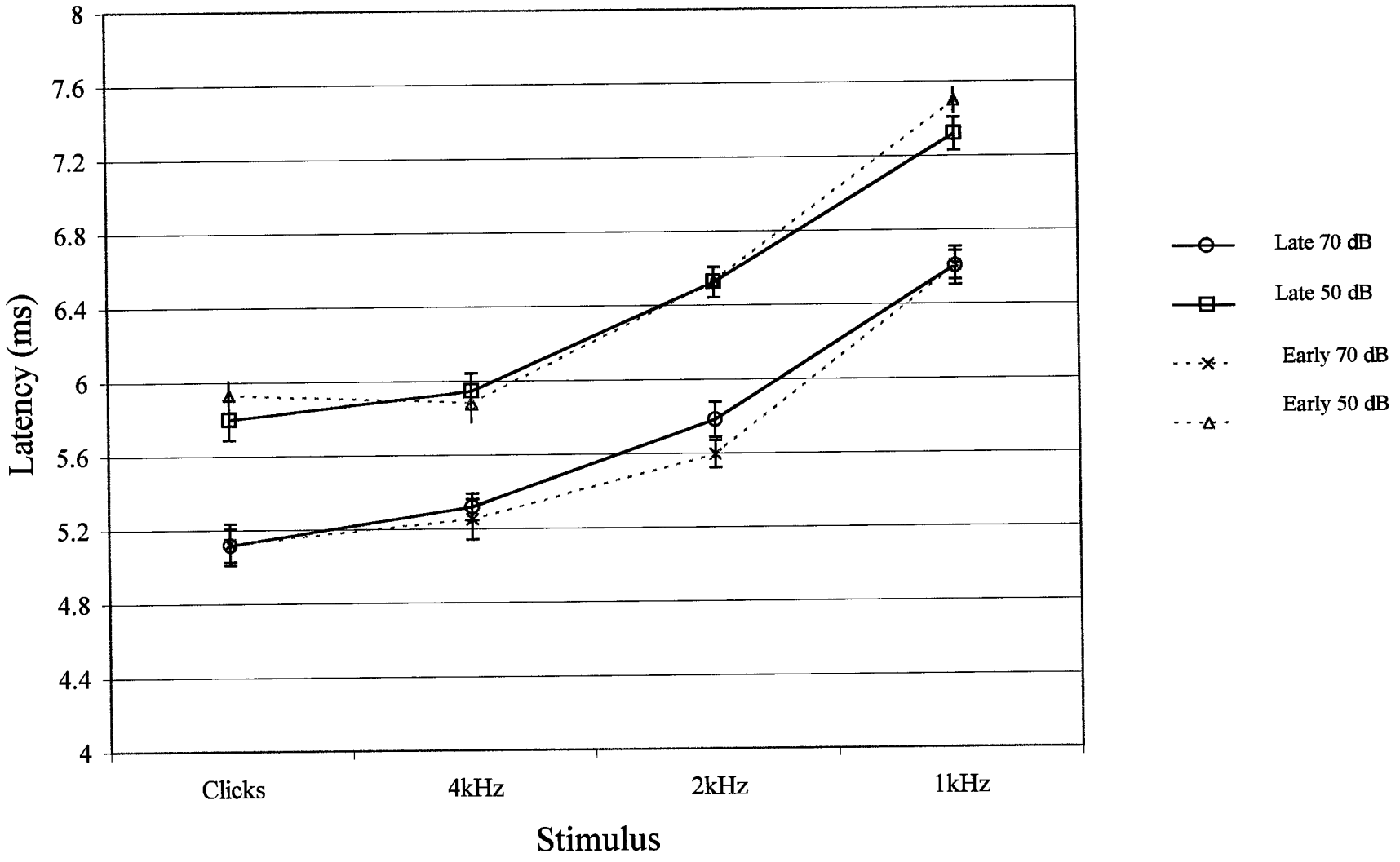


Figure 14. ABR Latencies in the Early and Late-Exposed Groups



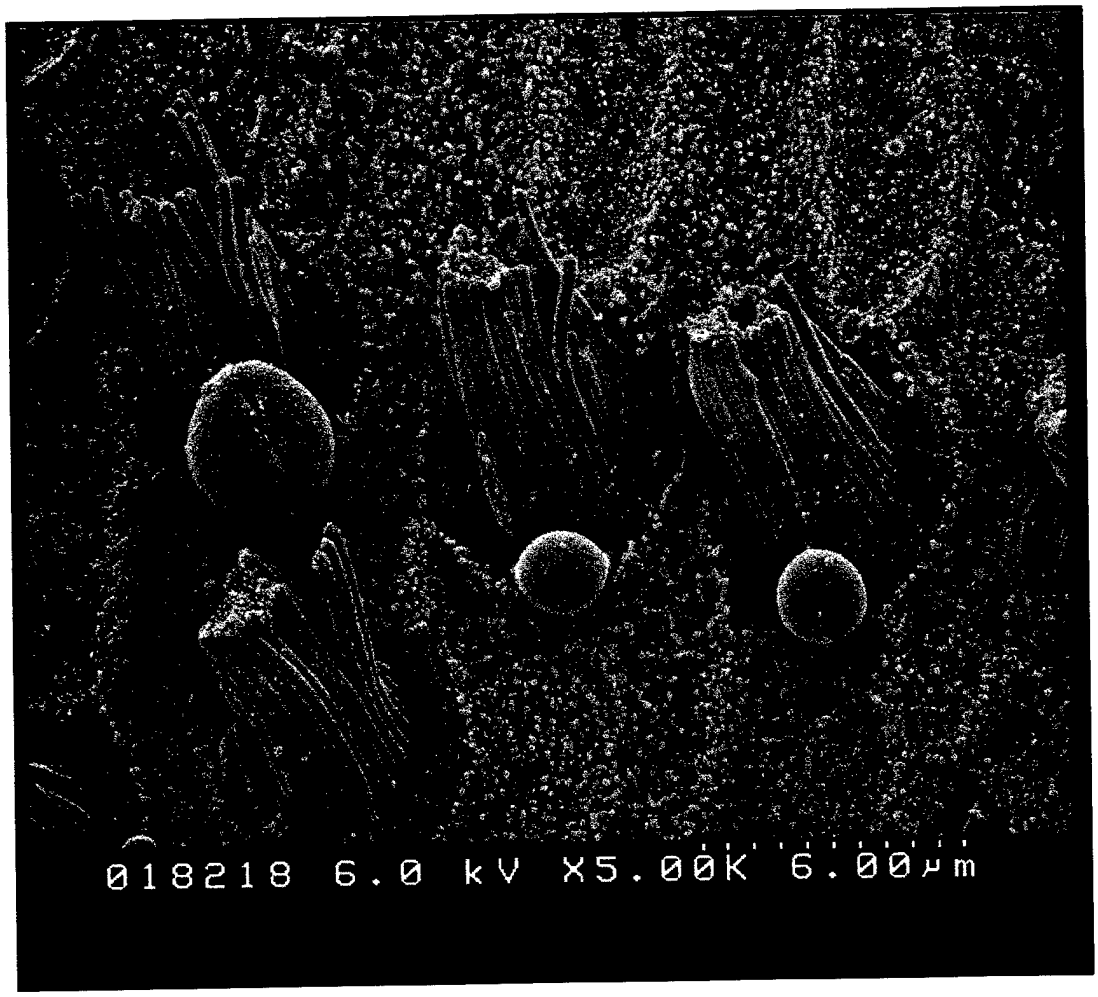


Figure 15. Arrows indicate cytoplasmic ballooning of outer hair cells from the apical portion of the cochlea of a noise-exposed fetal sheep.

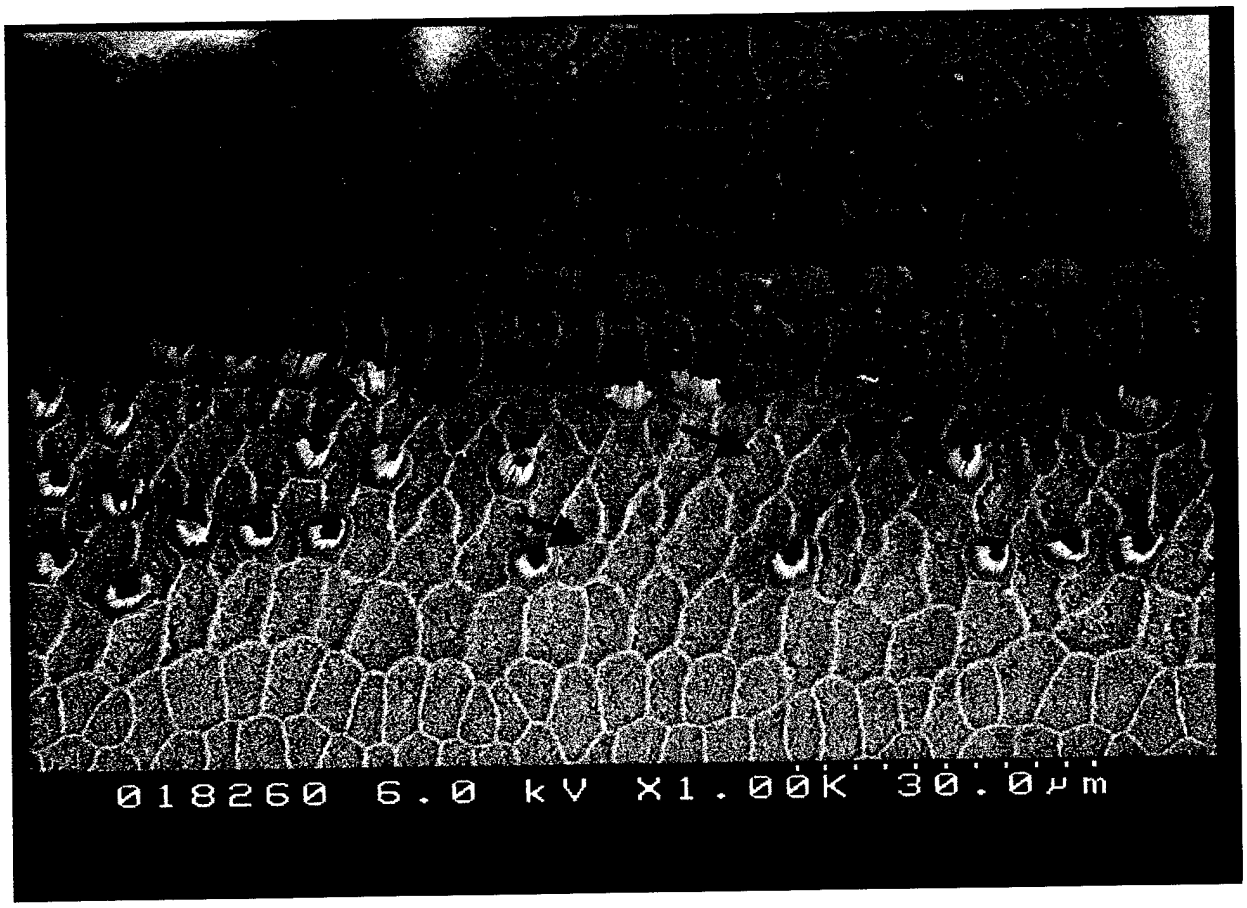


Figure 16. Arrows indicate locations of missing outer hair cell (OHC) in all three rows of the middle turn of a noise-exposed fetal sheep. Phalangeal scars have completely replaced the OHC.

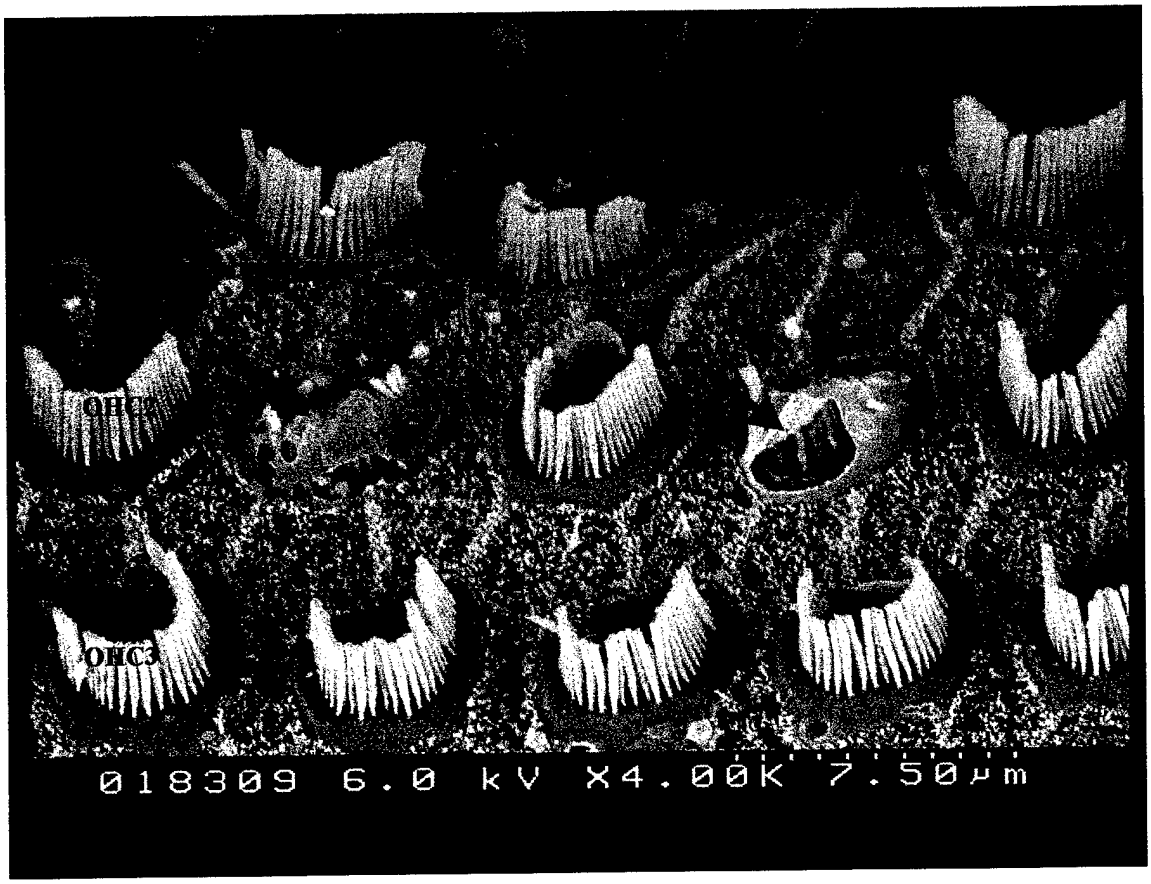


Figure 17. Arrows indicate a webbing-like material that has formed over the stereocilia of OHC2 of noise-exposed fetal sheep.

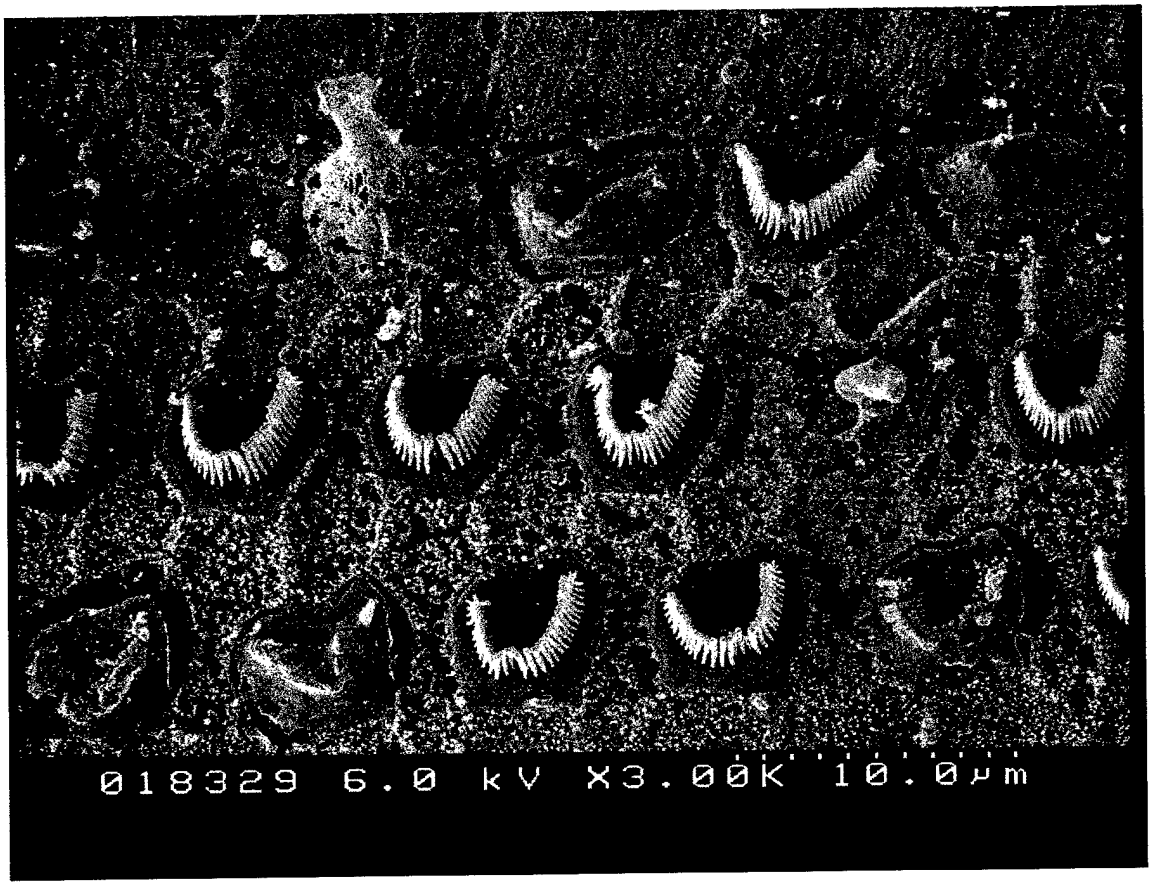


Figure 18. Stereocilia under the webbing-like material are almost completely dissolved (Arrows).

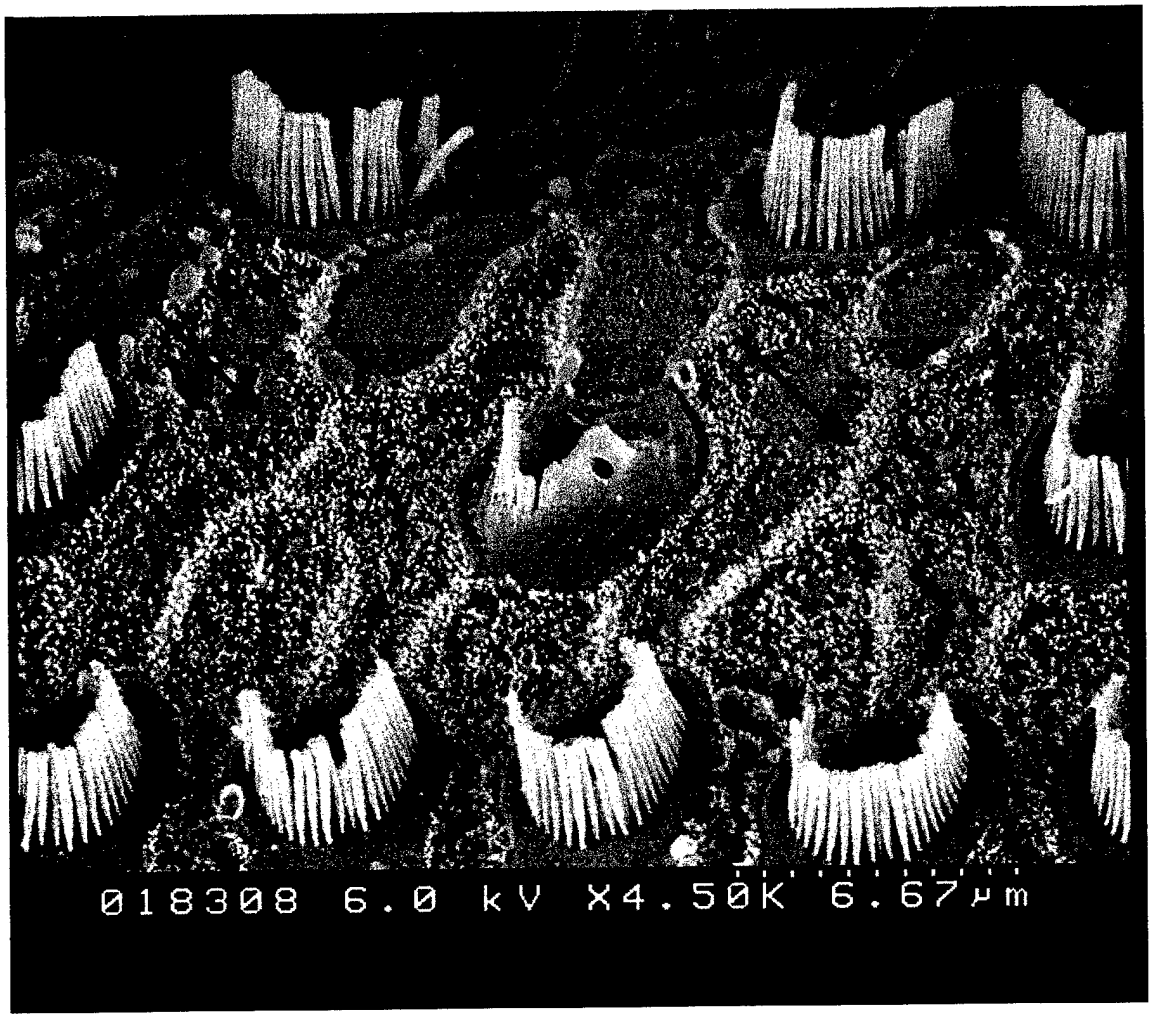


Figure 19. Hair cells in OHC2 are completely gone and have been replaced with phalangeal tissue (Arrows).

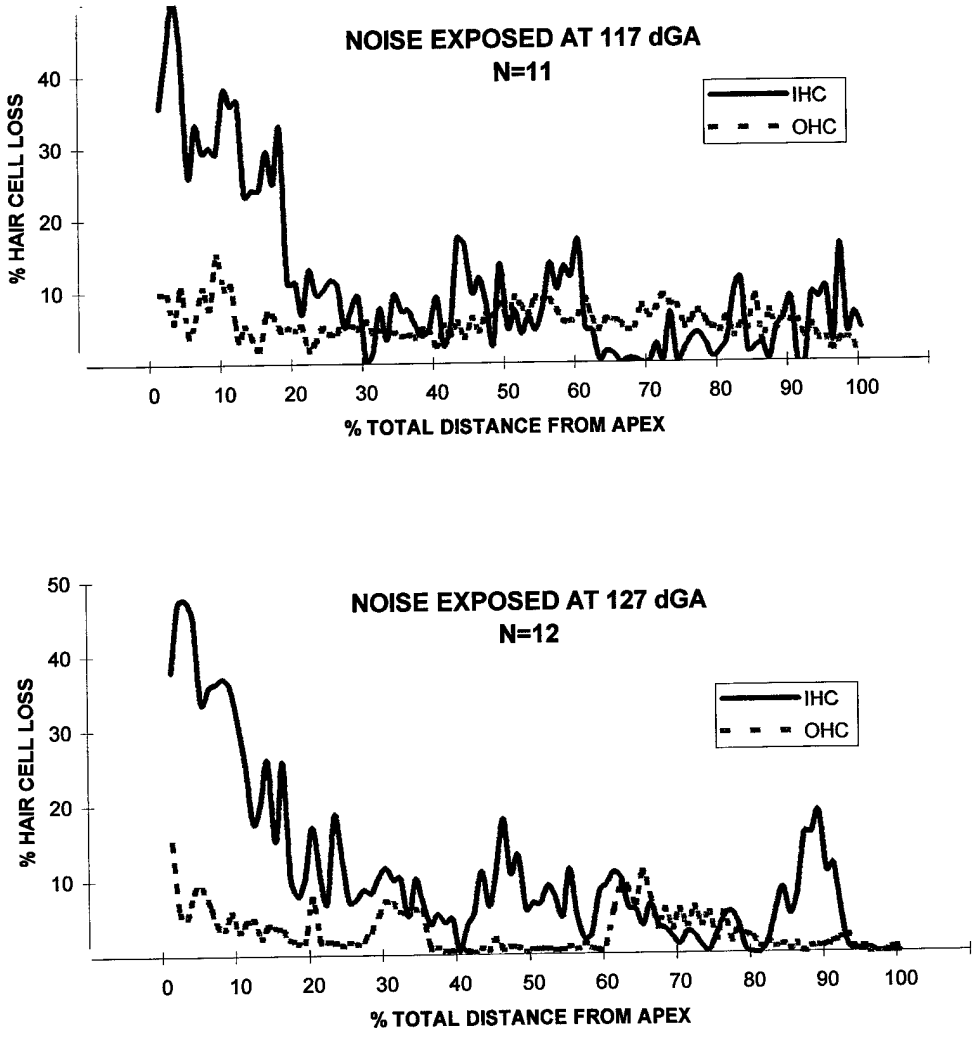


Figure 20. Average percentage of damage to inner (IHC) and outer hair cells (OHC) of fetal sheep cochleae. The cochleograms show hair cell integrity from fetuses exposed at 117 and 127 days gestational age (dGA) to 20 impulses at 169 dB peak sound pressure level. Controls were not exposed to noise.

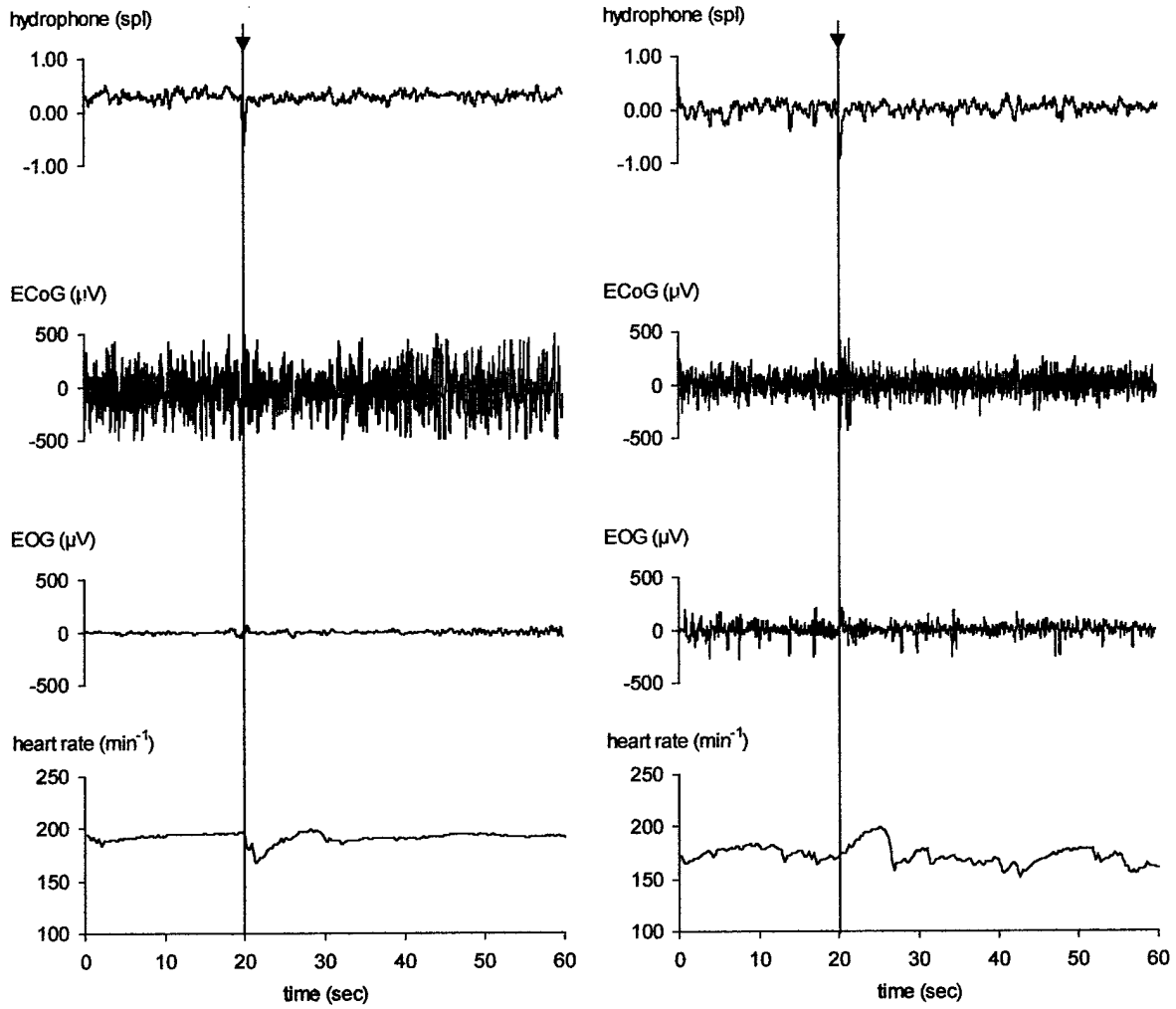


Figure 21. Typical examples of recordings of NREM sleep (left panel) and REM sleep (right panel) 20 seconds before, during and 40 seconds after impulse noise stimulation in a fetal lamb (animal O-97; 126 days gestational age; 5 days post-op). Recordings consist of intrauterine sound pressure levels, fetal ECoG, EOG, and instantaneous heart rate. Note that no visible changes in behavioral state occurred. In NREM sleep, a stimulus-related reduction of the ECoG amplitude was visible, combined with an abrupt but transient heart rate decrease. In REM sleep, a transient heart rate increase occurred. Arrows and vertical lines indicate occurrence of impulse stimulation.

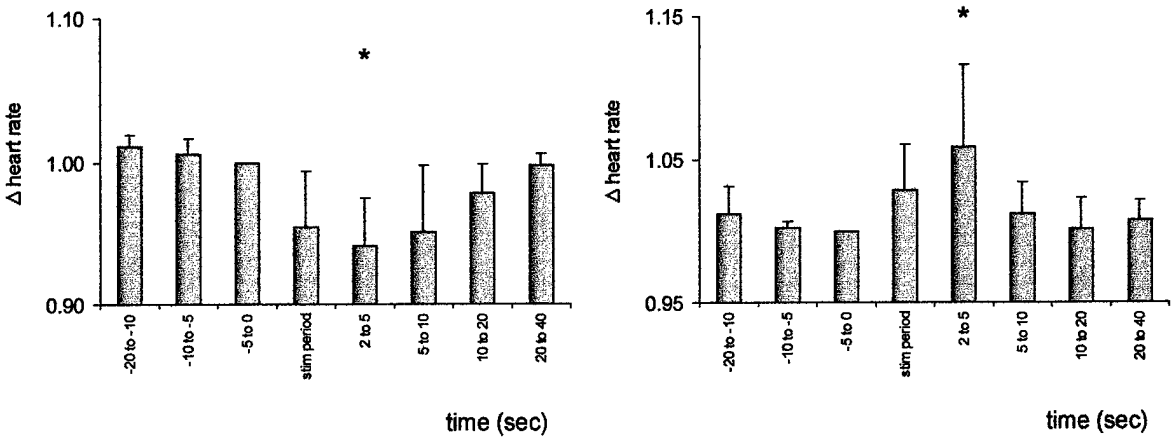


Figure 22. Changes in instantaneous heart rate due to impulse stimulation (stim period) during NREM sleep (left panel) and REM sleep (left panel) in a near-term fetal sheep (mean ± SD, * $p < 0.05$). Note the short but distinct decrease in fetal heart rate response in NREM sleep, but a significant increase during REM sleep.

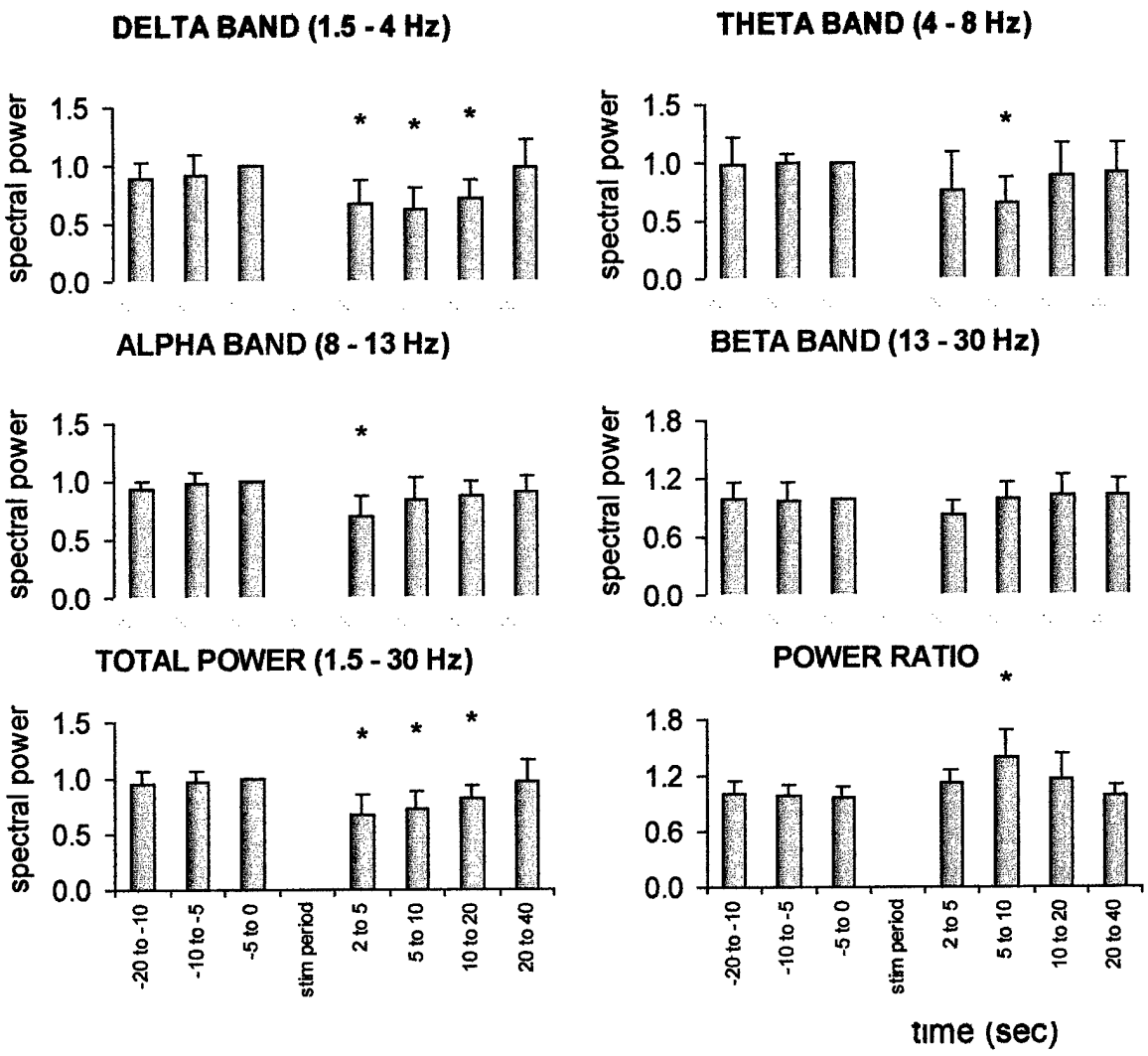


Figure 23. ECoG power in the standard frequency bands measured 20 seconds before and 40 seconds after impulse stimulation during NREM sleep (mean ± SD; * $p < 0.05$). Note the marked decrease in spectral Delta-, Theta-, and Alpha-band power resulting in decreased total power and an increase in the power ratio between higher and lower spectral bands.

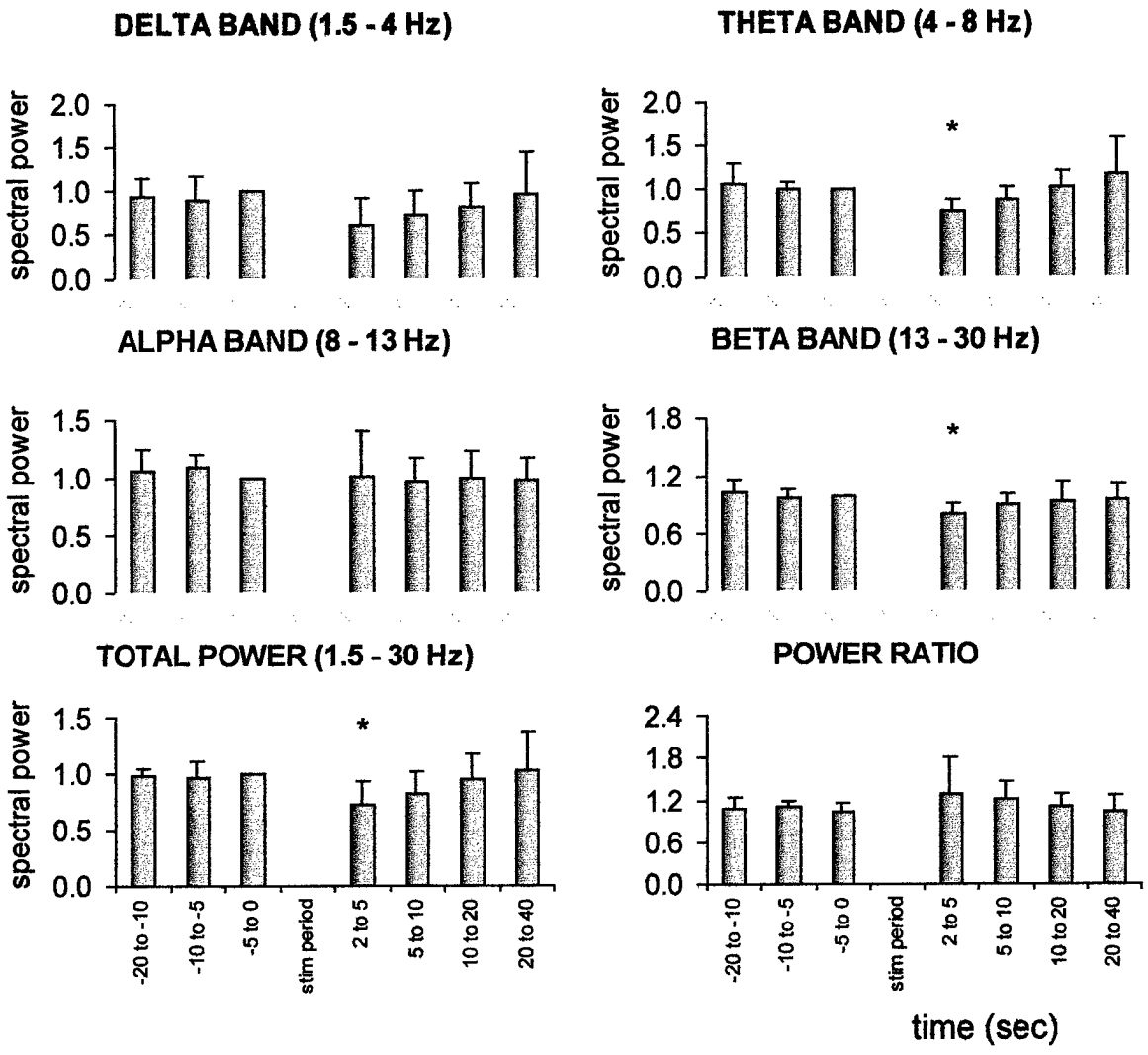


Figure 24. ECoG power in the standard frequency bands measured 20 seconds before and 40 seconds after impulse stimulation during REM sleep (mean ± SD; * $p < 0.05$). Note the mild and short decrease in spectral Theta- and Beta band-power resulting in decreased total power.

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DATE: September 17, 1999

TO: Commander, U.S. Army Medical Research and Materiel Command
ATTN: MCMR-RMI-S
504 Scott Street
Fort Detrick, MD 21702-5012

FROM: Kenneth J. Gerhardt, Ph.D.
Department of Communication Sciences and Disorders
Dauer Hall Room 338
P.O. Box 117420
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Gainesville, FL 32611-7420

RE: Final Report for Grant Number DAMD17-96-1-6302

Attached, please find the final report covering the period from **September 1, 1996 through August 31, 1999** for the grant titled "Impulse Noise Exposures: Characterization and Effects on Fetal Sheep in Utero." This report does not contain proprietary data and its distribution need not be limited. As indicated in the report, all work toward meeting the goals of the project is now completed.

One manuscript is approved for publication in Military Medicine, a second one is under review for Biology of the Neonate and a third is in preparation for Hearing Research. In addition, five abstracts funded by this grant have been published.

The following personnel have been supported by this grant:

Kenneth J. Gerhardt, Ph.D., P.I.
Robert M. Abrams, Ph.D., Co-P.I.
Scott K. Griffiths, Ph.D., Investigator
Sherri Anderson, Research Assistant
Isabelle Williams, Laboratory Technician
Ricardo Gauthier, Research Assistant
Katie Phelan, Research Assistant

If additional information is required, please let me know at your convenience.